Original Article

Immunochromatographic test versus microscopy in diagnosis of severe P. falciparum malaria

Ramrao Mundhe[1*], Prashant Patil[2], Maroti Karale[3], Shivraj Mane[4]

[1,3,4]Assistant Professor, Department of Medicine, Government Medical College, Latur, Maharashtra, INDIA.
[2]Professor and HOD, Department of Medicine, Government Medical College, Gondia, Maharashtra, INDIA.
Email: rammundhe7@gmail.com, sarthakpatil@yahoo.com, drmskarale13@gmail.com, shivumane9885@gmail.com

Abstract

Objective: Primary objective of this study was to compare diagnostic advantage of immunochromatographic test over peripheral blood smear microscopy in clinically suspected severe falciparum malaria. **Methods:** In this prospective study, total 125 clinically suspected severe malaria cases were enrolled during a period of two years from June 2014 to May 2016.All patients were subjected to peripheral blood smear microscopy and Paracheck Pf antigen detection test. **Results:** Total 180 cases were enrolled in study, of which 156 proved to be cases of severe plasmodium falciparum malaria diagnosed by either peripheral smear examination or Pf HRP-2 rapid malaria antigen test. Remaining 24 cases were excluded from the study. The sensitivity and positive predictive value of immunochromatographic Paracheck Pf test taking microscopy as reference standard was 91.28% and 95.10% respectively. **Conclusion:** HRP-2 based rapid diagnostic test for falciparum malaria has high sensitivity (91.28%) and positive predictive value (95.10%).

Keywords: Falciparum Malaria, peripheral smear microscopy, HRP 2, immmunochromatographic test.

[*]**Address for Correspondence:**
Dr. Ramrao Mundhe, Department of medicine, New building, Second Floor, Government Medical College, Latur, Maharashtra, INDIA.
Email: rammundhe7@gmail.com

Received Date: 08/11/2016 Revised Date: 12/12/2016 Accepted Date: 20/01/2017

Access this article online

Quick Response Code:

Website: www.medpulse.in

DOI: 25 January 2017

INTRODUCTION

Malaria is a public health problem in more than 90 countries. According to the latest estimates of WHO, released in December 2015, there were 214 million cases of malaria in 2015 and 438 000 deaths[1].In India, during 2011, the malaria incidence was around 1.31 million cases, 0.67 million P. falciparum (Pf) cases and 754 deaths while during 2014 (till October), 0.85 million cases, 0.54 Pf cases and 316 deaths have been reported[2]. Being associated with most serious complications, early diagnosis and prompt treatment is of paramount importance to reduce the mortality and morbidity and also for drug resistance associated with it. Microscopy remains the reference standard,[3]but this requires the availability of a good microscope, significant technical skills, good-quality reagents, clean slides and is difficult to maintain in remote and poorly resourced areas. So it requires the availability of a rapid, sensitive, and specific test at an affordable cost. Compared with microscopy, malaria rapid diagnostic tests (RDTs) do not require extensive training or well-maintained equipment. Malaria RDTs are immunochromatographic tests that identify malaria antigens, most commonly Plasmodium falciparum histidine rich protein-2(PfHRP2) or Plasmodium lactate dehydrogenase (pLDH)[4,5]. RDTs have been evaluated extensively for the diagnosis of uncomplicated malaria but not for severe malaria[6], and diagnostic test requirements are different in severe disease; e.g. a high sensitivity is of utmost importance, because missing a case may result in inappropriate treatment and death. We therefore compared the diagnostic performance of a commonly used PfHRP2-based RDT (Paracheck; Orchid Biomedical) with that of expert microscopy, which was used as the reference standard, for the diagnosis of severe malaria.

MATERIAL AND METHODS

This is an observational study conducted in medicine at Government Medical College, Latur over a period of two years from November 2014 to November 2016.Total 180 suspected severe malaria cases aged 12 years and more admitted in medicine wards of all units were included in the study. Ethics committee of the institution approved the study. Informed consent was taken from patient or

How to site this article: Ramrao Mundhe, Prashant Patil, Maroti Karale, Shivraj Mane. Immunochromatographic test versus microscopy in diagnosis of severe P. falciparum malaria. *MedPulse – International Medical Journal.* January 2017; 4(1): 154-156. http://www.medpulse.in (accessed 28 January 2017).

closest relative. Uncomplicated malaria cases, patients who have taken antimalarial treatment in previous four weeks were excluded from study. Each patients baseline data, age, demography were recorded. A detailed history was taken followed by a detailed clinical examination to assess clinical severity and complications of malaria. All patients were subjected to peripheral smear microscopy and Paracheck Pf antigen detection test (immunochromatographic test) which were done by two separate individuals and none had idea about the result of other test. For diagnosis of severe malaria, other laboratory investigations carried out were complete blood count, liver function tests, kidney function tests, random blood sugar, arterial blood gas, chest x-ray and ultrasonography.

Statistical analysis

Data was analyzed using Epi - info 3.4.3 statistical software. Validity measures like sensitivity and positive predictive value were calculated using MS Excel. Chi square test was used for testing significance of difference between two proportions. P- Value < 0.05 was considered as statistically significant.

RESULTS

Total 180 cases of suspected severe malaria were enrolled for the study. Of the 180 cases 156 cases proved to be the cases of severe malaria produced by plasmodium falciparum infection diagnosed on Peripheral smear examination or by antigen detection test. The remaining 24 cases were excluded from the study either due to negative tests for both RDT and microscopy or species variation on peripheral blood smear. Total 156 patients diagnosed to have severe malaria were recruited in the study, of which 90 were males and 66 were females constituting 57.69% and 42.31% respectively, with a male to female ratio of 1.36:1.The number of patients from rural area and urban area were 95 (60.90%) and 61(39.10%) respectively. The mean age of patients was 32.72 ± 13.42 years and the most common age group affected was 20-39 years comprising 55.18% of cases. Among 156 cases microscopy was positive in 149 (95.51%) patients and Paracheck Pf test was positive in 143 (91.67%) patients as shown in table 1 and 2. The relationship between these tests is shown in Table no. 3.

Table 1: Showing PBS positivity for falciparum malaria

PBS for MP	No. of cases	Percentage (%)
Positive	149	95.51%
Negative	07	4.49%
Total	**156**	**100%**

Peripheral blood smear was positive in 95.51% of patients and was negative in 4.49% of patients.

Table 2: Showing Pf HRP-2 Antigen test positivity

Pf HRP-2 Ag	No. of cases	Percentage (%)
Positive	143	91.67 %
Negative	13	8.33 %
Total	**156**	**100 %**

PfHRP-2 Antigen test was positive in 91.67% of patients and was negative in 8.33% of patients.

Table 3: Showing relationship between PBS for MP and Pf HRP-2 Ag test

	PBS for MP Positive	PBS for MP Negative
Pf HRP-2 Positive	136	07
Pf HRP-2 Negative	13	00

Sensitivity and positive predictive value were calculated taking PBS for MP as a reference standard and found to be 91.28% and 95.10% respectively. Among 156 cases screened for P. falciparum infection with both RDT and microscopy, 136 (87.18%) of the cases were found to be positive for P. falciparum with both methods (Table 3).

DISCUSSION

Routine malaria diagnosis is based on detection of asexual parasite stage in the stained blood smears using microscopy or detection of parasite antigen using RDTs. The present study has compared the performance of Paracheck-Pf test against the gold standard microscopy in febrile patients diagnosed as severe malaria based on either test. Total 156 patients diagnosed to have severe malaria were recruited in the study, of which 90 were males and 66 were females constituting 57.69% and 42.31% respectively, with a male to female ratio of 1.36:1.The number of patients from rural area and urban area were 95 (60.90%) and 61(39.10%) respectively. The mean age of patients was 32.72 ± 13.42 years and the most common age group affected was 20-39 years comprising 55.18% of cases. Among 156 cases microscopy was positive in 149 (95.51%) patients and Paracheck Pf test was positive in 143 (91.67%) patients as shown in table 1 and 2 respectively. Both the tests for severe malaria were positive in 136 (87.18%) of the patients (Table 3).Sensitivity and positive predictive value of Paracheck Pf HRP-2 based RDT was found to be 91.34% and 94.05% respectively (Table 3). Our finding of sensitivity and positive predictive value was compared with other studies as shown in following Table 4.

Table 4: Comparison of sensitivity and PPV with other studies

Studies	Sensitivity (%)	PPV (%)
Zinaye Tekeste *et al*[8]	95.2	94.8
Awwal A, *et al*[9]	91.42	90.56
Uddin MM, et [10]	94.1	100
Hussein M, *et al* [11]	94.1	93.3
Sani *et al*[12]	90.2	93.0
Tekola Endeshaw *et al*[13]	47.5	56.8
Present study	**91.28**	**95.10**

Sensitivity (91.28%) and positive predictive value (95.10%) of Paracheck Pf HRP-2 based RDT found in our study corresponds with findings of Zinaye Tekeste *et al*,[8]Awwal A, *et al*,[9] Uddin MM, *et al*,[10] Hussein M, *et al*,[11] Sani *et al*.[12]Reports from all these studies indicated that RDTs have a comparable level of accuracy to microscopy in clinical settings. But study done by Tekola Endeshaw[13] *et al* show much lower sensitivity and positive predictive value of the RDTs than predicted by present study. This variation may be due to difference in sample size, population selection method and theRDT kits used. Also, it is possible that parasitaemias were different in these study groups bases on endemicity of malaria.The diagnostic accuracy of RDTS can be affected by several factors such as quality of the products, storage temperature and humidity, and end users' performance also. Hence in spite of high sensitivity and positive predictive value.[14] Out of the 156 patients, 7 (4.49%) cases were positive for Parackeck pf antigen test but were negative by peripheral blood smear microscopy. Reason for this could be prior antimalarial treatment, as it affects the malaria parasite count in peripheral blood film and can cause negative malarial microscopy. But prior treatment doesn't affect the result of Paracheck Pf test as it detects the HRP-2 protein not the parasite itself.[15] In this study, 8.33 %(13/110) patients were positive for peripheral blood smear but negative by Parackeck pf antigen detection test. This could be due to low sensitivity of RDTs below the level of 100 parasites per micro liter compared to microscopy as is one of the drawbacks of RDTs[5, 16]with parasitaemia below the RDT's threshold detection level. Thus, it can be deduced from here that based on high sensitivity and positive predictive value, diagnostic capability of HRP-2 based immunonochromatographic test (RDTs) is useful in resource poor settings but peripheral smear microscopy still remains the gold standard for diagnosis of falciparum malaria. Hence in settings where microscopy is unavailable, using RDT can lead to a significant reduction in the over prescription of anti-malarial drugs.

CONCLUSION

HRP-2 based rapid diagnostic test for falciparum malaria has high sensitivity (91.28%) and positive predictive value (95.10%). This test is useful where malarial microscopy is not reliable or unavailable and where patients have received antimalarial treatment.

REFERENCES

1. WHO, Fact sheet no. 94, recent advances in research and development of malaria, April 2015.
2. Govt. of India (2015), Malaria, Magnitude of the problem NVBDCP, DGHS, Ministry of Health and Family Welfare, New Delhi.
3. World Health Organization (WHO). Guidelines for the treatment of malaria Geneva, Switzerland: WHO, 2010.
4. World Health Organization (WHO). Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: round 2 (2009). Geneva, Switzerland: WHO, 2010.
5. Moody AH, Joshi SS, Joshi SR. Newer advances in malaria diagnosis. Postgraduate medicine 2000;14:17-33.
6. Birku Y, Welday D, Ayele D, Shepherd A. Rapid diagnosis of severe malaria based on the detection of Pf-Hrp-2 antigen. Ethiop Med J 1999; 37:173– 9.
7. WHO Guidelines for the treatment of malaria. Geneva, Switzerland, World Health Organization; Third edition. April 2015.
8. Zinaye Tekeste, Meseret Workineh, Beyene Petros. Comparison of Paracheck Pf test with conventional light microscopy for the diagnosis of malaria in Ethiopia. Asian Pacific Journal of Tropical Disease 2012:1-3.
9. Awwal A, Amin MR, Hafiz SAMMA, Rahman M, Ali M, Sattar A, Rahman MR, Faiz MA. Immunochromatographic test over microscopy in diagnosis of severe malaria in Bangladesh. JAFMC Bangladesh 2011 June;7(1):4-7.
10. Uddin MM, Hussain SMB, Rahman MM, Hossain MD. Effectiveness of immuno-chromatographic test in the diagnosis of falciparum malaria. JAFMC Bangladesh. 2011 June;7(1):31-33.
11. Hussein Mohammed, Moges Kassa, Amha Kebede, Tekola Endeshaw. Paracheck-pf test versus microscopy in the diagnosis of falciparum malaria in Arbaminch Zuria Woreda of South Ethiopia. Ethiop J Health Sci. 2012 July;22(2):93-98.
12. Sani UM, Jiya NM, Ahmed H. Evaluation of a malaria rapid diagnostic test among febrile children in Sokoto, Nigeria.eInt. J. Med.Med.Sci 2013.
13. Tekola Endeshaw *et al*. Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. Malaria Journal 2008, 7:118 doi:10.1186/1475-2875-7-118.
14. World Health Organization: The role of laboratory diagnosis to support malaria disease management: Focus on the use of rapid diagnostic tests in areas of high transmission, report of a WHO technical consultation, 25-26 October 2004. Geneva, Switzerland, World Health Organization; 2008:4-48.
15. Desakorn V. Silamut K, Angus B, *et al*. Semi-quantitative measurement of P. falciparum antigen Pf HRP 2 in blood adn plasma. Trams R Soc Trop Med Hyg 1997, 91: 479 83.
16. Moonasar D, Goga AE, Frean J, Kruger P, Chandramohan D: An exploratory study of factors that affect the performance and usage of rapid diagnostic tests for malaria in the Limpopo Province, South Africa. Malar J 2007, 6:74.

Source of Support: None Declared
Conflict of Interest: None Declared