SIMPLE YET VALUABLE – ANTIGEN DETECTION ICT (IMMUNO CHROMATOGRAPHIC TEST) IN EARLY DIAGNOSIS AND TREATMENT OF COMPLICATED MALARIA IN ENDEMIC AREAS

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ABSTRACT

Introduction : Severe thrombocytopenia and acute renal failure, once considered to be exclusively due to P. falciparum were observed recently in P.vivax infection also. Early diagnosis and appropriate treatment is the basis to prevent complications. Diagnosis , solely based on clinical symptoms is unreliable. Microscopist is not available everywhere and reliability is also questionable. So a simple alternative test is required for the present situation.

Aim: The present study is to compare microscopy with Ag detection ICT during an epidemic and to document ARF and thrombocytopenia with P.vivax infection in endemic area.

Materials and methods: 78 patients were screened for malaria during the epidemic from December 2009 and January 2010. Malaria Ag detection ICT was used alongside the gold standard microscopy.

Result: Total patients screened = 78 .Positive by thick smear : 23 (30%) i.e., P.vivax-20(26%), P.falciparum 3(4%) Positive by Ag detection ICT : 34 (44%) i.e, P.vivax22(28%) P.falciparum12(15%) Sensitivity (ICT) 95.83%, Specificity (ICT) 79.82%, Thrombocytopenia : P.vivax – 7 out of 22 (32%)P.falciparum – 7 out of 12 (58%) Acute renal failure – P.vivax -2 out of 7, P.faciparum – 1 out of 12 . Both the tests became negative in 6 patients (1 P.falciparum & 5 P.vivax) who came for follow up in a week period.

Conclusion:- P.vivax also should be considered in patients with severe thrombocytopenia and acute renal failure. Employing rapid Ag detection ICT in endemic areas will reduce the indiscriminate use of anti malarial drugs and drug resistant malaria. It should be confirmed by microscopy. A more sensitive method holds justice , when microscopy and expertise are not available.

Key words: *acute renal failure , thrombocytopenia, P.vivax , epidemic, endemic, microscopy, Ag detection ICT*

INTRODUCTION

Recently it was observed that many patients with malaria due to Plasmodium vivax also develop thrombocytopenia and acute renal failure, once considered to be exclusively due to Plasmodium falciparum infection[1,2,3,4]. The major problem in controlling the morbidity and mortality is due to lack of infrastructure and limited access to early diagnosis and treatment particularly in endemic areas . This is more challenging during epidemics. Diagnosis solely by symptoms is unreliable[5,6]. Inappropriate use of anti malarials causes toxicity and development of resistance. Microscopy is simple but needs expertise and reliability is questionable especially at low parasitemia and in the interpretation of mixed infection[7,8]. A simple and cost effective alternative to microscopy is the need of the day to overcome these deficiencies[9,10].

AIM

With the above background, the present prospective study was conducted in an endemic area during a fever epidemic to compare microscopic examination of blood film with simple antigen detection assay by ICT for early diagnosis and to document the association of severe thrombocytopenia and acute renal failure due to P.vivax infection .

MATERIALS AND METHODS

During the last fever epidemic between December 2009 and January 2010, a total of 78 patients who came with fever to a multi specialty referral hospital , were screened for malaria. Informed consent was taken from all patients. Blood samples were collected in EDTA coated tubes before the administration of anti malarials which may interfere with the detection of parasites due to its morphologic alteration. Both thick and thin smears were prepared soon after collection which enables better adherence of films to the slide and causes minimal distortion of parasites and red cells. A commercial one

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step malaria antigen rapid test (SD Bioline, Manufactured by Bio Standard Diagnostics Pvt.Ltd,Plot No:266,Sector-6, IMT Manesar, Gurgaon-122050,Haryana,India) was used alongside the gold standard (Leishman stained film)microscopy. The SD Bioline malaria antigen Pf/Pan test is an immunochromatographic (ICT - rapid) method for the qualitative detection of antigens specific to P. falciparum and P. vivax simultaneously in human whole blood .

The test cassette contains a membrane strip, which is pre coated with one monoclonal antibody (specific to the histidine rich protein II "HRP II"of P.falciparum) and the other polyclonal antibody (pan specific to the lactate dehydrogenase "LDH" of all Plasmodium species P.faliparum, P.vivax, P.malariae, P.ovale] as two separate lines across the strip.

Procedures were followed strictly as per the manufacturer's standard operating manual. Both tests were repeated only in 6 cases who came for follow up to evaluate the efficacy of treatment. Along with routine urine and blood screening tests , platelet count , renal parameters and tests to rule out other causes of fever were done as per the standard procedures .

RESULT

Of the 78 patients screened for malaria by peripheral smear, 23patients (30%) were positive. Of this, 20 patients (26 %) were positive for P.vivax and 3 patients (4%)were positive for P.falciparum .

Whereas by antigen detection ICT,34 patients (44%) were positive. Of this, 22 patients(28%) were positive for P.vivax and12 (15%) were positive for P.falciparum. Hence 9 cases(11%) were detected additionally for P.falciparum .

Of these, 22 patients (28%) were positive by both peripheral smear and antigen detection by ICT. Considering microscopy as gold standard , the sensitivity and specificity of antigen detection ICT were 95.83% and 79.82% respectively.

Out of 34 patients positive by antigen detection ICT, 14 patients (41%) had platelet count less than one lakh. Of the 22 P. vivax cases ,7patients (32%) had thrombocytopenia. In 2 cases, the count was between 20000 to 50000.

In the remaining 5 patients it was between 50000 to 100000. Among the 12 P. falciparum cases ,7 patients (58%) had thrombocytopenia. Acute renal failure was detected in 2 persons with P.vivax infection and in one P. falciparum positive patient . Antigen detection was positive as early as 5th day of fever. Both the tests became negative in 6 patients (1 P.falciparum & 5 P.vivax) in a week period who came for follow up.

Test	Thick smear		Total
	Positive	Negative	
ICT Positive	23 (a)	11 (b)	34
ICT Negative	01 (c)	43 (d)	44
Total	24	54	78

Table : 1 – Microscopy vs ICT

$$Sensitivity = \frac{a}{a+c} \times 100 = 95.83\ \%$$

$$Specificity = \frac{d}{b+d} \times 100 = 79.62\ \%$$

$$Positive\ predictive\ value = \frac{a}{a+b} \times 100 = 52.27\%$$

$$Negative\ predictive\ value = \frac{d}{c+d} \times 100 = 97.72\%$$

DISCUSSION

It is estimated that there are more than 50 million cases and 1.1-2.2 million deaths due to malaria every year[11]. Its occurrence is noted in more than 90 countries[12]. Approximately, 2.48 million malaria cases are reported annually from South Asia, of which 75% cases are contributed by India alone[13].

Malaria also accounts for the recent battle of mosquito borne mysterious fever epidemic in southern districts of Tamil Nadu. Diagnosis of malaria is much challenging especially during epidemics. Most of the patients from rural area came with fever and low platelet count. They were referred to city referral hospitals for further management. Since this sudden influx of patients arose from different rural corners, the city referral hospitals became overcrowded. Single room was being shared by two patients, in some hospitals even the dormitory and labor rooms were also converted as wards. The situation was still worsened to as many patients thronged in waiting list for admission. Most of these patients required two to three units of platelet transfusion to

prevent complications. Another constraint on this is that, most of the blood banks do not have platelet separation facility, except for one or two to fulfill the needs of these patients.

More over, platelet transfusion requires fresh blood. Amidst this emergency, every patient required three donors .Platelet separation is a laborious procedure and it takes as long as three hours. Much worrisome is that one does not know how many donors were in window period for transfusion transmitted virus (TTV) or yet to be identified infective agents. This was the situation during epidemic.

When can one suspect that someone has the disease "malaria"? No single clinical sign predicts the diagnosis of malaria. Yet malaria must always be considered in cases of fever in the tropics. Since the symptoms can be quite diverse, a clinical diagnosis alone is unreliable. Moreover, rapid detection and effective treatment is a pre-requisite for reducing the morbidity and mortality due to malaria.

Microscopic examination of blood smears is the widely used routine method for detection of malaria parasite and remains the gold standard for malaria diagnosis[14]. But microscopic examination is time consuming and requires considerable technical expertise for its interpretation and of limited sensitivity at low levels of parasitaemia[15]. The result also depends upon the quality of microscope, staining, the technique with which blood film is prepared and also on the concentration and motivation of the microscopists[16,17]. Several studies have shown that the ability to diagnose malaria by blood film examination alone is about 75% for P. falciparum[18,19,20].

Besides these, majority of malaria cases occur in rural areas where there is little or no access to reference laboratories and in many areas expert microscopist is not available. The urgent need for a simple and cost effective test to overcome the deficiencies of light microscopy has been recognized for a longtime[9,10].

In the present study, 30% were positive by thick peripheral smear microscopy. Antigen detection by ICT was 44% .Considering peripheral smear as gold standard the sensitivity of ICT was 95.83 and specificity was 79.62%. Possible reasons for the high sensitivity of ICT method are the detection of both HRPII and LDH and their performance ability even in low parasitemia. It detects malarial infection even on the 5th day of illness since this is the earliest period the patients in the present study turned up to the hospital . Repeat testing in 6 cases who came for follow up became negative in a week period by both methods .This implies that ICT can be used as a tool to assess the efficacy of treatment.

By Ag detection ICT 12 (15%) cases were positive for P.falciparum when compared to 3(4%) cases by peripheral smear. It may be well explained that in patients with P.falciparum malaria, sometimes the parasites can be sequestered and are not present in peripheral blood. Thus a P. falciparum infection could be missed due to the absence of parasites in a blood film[12]. But the Ag detection ICT method is still useful in this situation.

When compared to microscopy 9 cases were detected additionally for P.falciparum by antigen detection ICT. Potential causes for PfHRP2 positivity, include gametocytemia, persistent viable asexual-stage parasitemia below the detection limit of microscopy (possibly due to drug resistance), delayed clearance of circulating antigen(free or in antigen-antibody complexes), persistence of antigens due to sequestration and incomplete treatment .

But false positive tests can occur with antigen detection ICT for many reasons such as cross reaction with non-P.falciparum malaria or rheumatoid factor. Proportion of persistent positivity has been linked to the sensitivity of the test, type of the test, degree of parasitemia and possibly the type of capture antibody[21].

On the other hand, false negative tests have been observed even in severe malaria with parasitemias >40000 parasites/µl. This has been attributed to possible genetic heterogeneity of PfHRP2 expression, deletion of HRP-2 gene, presence of blocking antibodies for PfHRP2 antigen or immune-complex formation, prozone phenomenon at high antigenemia or to unknown cause[21].

Therefore, in cases of suspected severe malaria or complex health emergencies, a positive result may be confirmatory but a negative result may not rule out malaria. So a negative ICT result should always be confirmed by microscopy.

It should be emphasized that P. falciparum malaria, a potentially lethal disease, must not be missed because of a false-negative test.

Though the thick smear study is considered as gold standard, the interpretation is prone for subjective variation even among experienced microscopists . In the present study ,even with this small number of samples, variation was experienced in the interpretation of smears between microscopists and we solved this by getting opinion from another microscopist.

Although, severe complications including profound thrombocytopenia are common due to P.falciparum[22,23.24]. but now, we are facing such complications due to P.vivax . It is contrary to the belief that vivax infection is benign. The association of platelet depletion and P.vivax has been observed by various workers[3]. Unlike in the past, incidence of severe thrombocytopenia and acute renal failure due to P. vivax are increasing in endemic areas[25] which may be due to a new genotype of P. vivax. A study from Mumbai has reported six cases of P.vivax with thrombocytopenia, where the level of platelets ranged from14000 to 92000/µl[25]. A recent study from India has reported cerebral malaria as a complication of P.vivax in 5 out of 40 cases[26,27].

Thrombocytopenia seen in complicated P. falciparum is due to disseminated intravascular coagulation along with platelet endothelial activation, but the one seen in uncomplicated malaria like P. vivax has multi factorial etiology. Few postulated mechanisms are macrophage activation leading to platelet destruction, increased levels of cytokines, immunological destruction due to antiplatelet IgG, oxidative stress, shortened platelet life span in peripheral blood, sequestration in non splenic areas and psuedothrombocytopenia due to clumping of platelets[28,29,30,31,32].

In our study the platelet count was lower than normal in 7 out of 20 P.vivax cases. In 2 cases, the count was between 20000 to 50000. In the remaining 5 cases it was between 50000 to 100000. In two cases of P. vivax infection acute renal failure was documented with high urea and creatinine. The ARF and thrombocytopenia were reverted to normalcy with effective anti malarial and supportive therapy.

As per national antimalarial drug policy all fever cases, both in high and low risk areas, without any other obvious causes are to be treated with antimalarials[33]. Even in Integrated Management of Childhood Illness (IMCI) strategy, all fever cases are given antimalarials[34]. This indiscriminate use of antimalarials, is constantly increasing the overall drug pressure.

The greatest decrease in antimalarial drug use could be achieved through improving the diagnosis of malaria at least in places where diagnostic facilities are available. With the development of rapid antigen tests which are cost effective, simple, easy, user friendly and does not require expertise ,considerable reduction of unnecessary treatment is possible in resource poor settings[13].

CONCLUSION

The present study suggests in patients with acute renal failure and severe thrombocytopenia particularly in endemic areas, P.vivax infection as etiology should also be kept in mind. Instead of prescribing blind anti malarial therapy for all fever cases during epidemics, simple rapid antigen detection ICT may be a good choice for early diagnosis in endemic areas where there are limited access to reference laboratory. It will reduce the indiscriminate use of anti malarial drugs ,thereby the evolving drug resistant malaria. Microscopy may be used for confirmation if and when available. Though false positive is possible with ICT , for the purpose of screening, a more sensitive method hold justice, particularly when microscopy and expertise are not available. At the same time if there is a strong clinical suspicion but antigen detection ICT is negative , the gold standard microscopy is a must, because the potential serious malarial infection should not be missed.

This study implies that ICT is a valuable adjunct at the time of emergency for rapid diagnosis, although microscopy remains the mainstay for the diagnosis of malaria for routine use in countries like India. However microscopy is much useful to assess parasite burden and its morphological stages.

REFERENCES

1. Kakar A, Bhoi S, Prakash V, et al. Profound thrombocytopenia in Plasmodium vivax malaria. Diagn Microbiol Infect Dis 1999;35:243–4.
2. Makkar RP, Mukhopadhyay S, Monga A, et al. Plasmodium vivax malaria presenting with severe thrombocytopenia. Braz J Infect Dis 2002;6:263–5
3. Anju Aggarwal, Suman Rath, Shashiraj. Plasmodium vivax Malaria Presenting with Severe Thrombocytopenia. J Trop Pediatr 2005;51:120–1.
4. Prakash J, Singh AK, Kumar NS et al. Acute renal failure in Plasmodium vivax malaria. J Assoc Physicians India 2003;51:265–7.]
5. New Perspectives Malaria Diagnosis WHO/CDS/RBM/2000.14 Report Of A Joint Who/Usaid Informal Consultation 25–27 October 1999 WHO/MAL/2000.1091
6. Jamshaid Iqbal, Nabila Khalid, and Parsotam R. Hira Comparison of Two Commercial Assays with Expert Microscopy for Confirmation of Symptomatically Diagnosed Malaria Journal Of Clinical Microbiology, Dec. 2002, Vol. 40, No. 12 p. 4675–4678]
7. Malcolm Molyneux, Ray Fox Diagnosis and treatment of malaria in Britain BAQ1993;36:1 175-80
8. Management of Uncomplicated Malaria and the Use of Antimalarial Drugs for the Protection of Travellers Report of an Informal Consultation Geneva, 18 - 21 September 1995 World Health Organization WHO/MAL/96.1075 Rev.1 (1997)]
9. WHO Informal Consultation on Recent Advances in Diagnostic Techniques and Vaccines for Malaria1 A rapid dipstick antigen capture assay for the diagnosis of falciparum malaria* Bulletin of the World Health Organization, 1996, 74 (1): 47-54

10. Zaccheaus Awortu Jeremiah , Emmanuel Kufre Uko, Fiekumo Igbida Buseriand Theresa Awortu Jeremiah Field Evaluation of SD Bioline Rapid Malaria Diagnostic Test among Asymptomatic Malaria Infected Children in Port Harcourt,Nigeria Research Journal Parasitology 2 (1);39 -44,2007.]
11. Lal S, Dhillon GP, Aggarwal CS. Epidemiology and control of malaria Indian J Pediatr. 1999 Jul-Aug;66(4):547-54.].
12. Parija SC, Dhodapkar R, Elangovan S, Chaya DR. A comparative study of blood smear, QBC and antigen detection for diagnosis of malaria. Indian J Pathol Microbiol 2009;52:200-2 Ritabrata Kundu]
13. Nupur Ganguly ,Tapan Kr. Ghosh,Panna Choudhury,Raju C. Shah Diagnosis and Management of Malaria in Children Recommendations and IAP Plan of Action Indian Pediatrics Volume 42 November 17, 2005 1101 -1114]
14. Angela Hunt Cooke, Peter L. Chiodini, Tom Doherty, Anthony H. Moody, Junita Ries, and Margaret Pinder Comparison of a parasite Lactate Dehydrogenase–based immunochromatographic antigen detection assay (Optimal) with microscopy for the detection of malaria parasites in human blood samples Am. J. Trop. Med. Hyg., 60(2), 1999, pp. 173–176
15. O. E. Lema, J. Y. Carter, N. Nagelkerke, M. W. Wangai, P. Kitenge, S. M. Gikunda, P. A. Arube, C. G. Munafu, S. F. Materu, C. A. Adhiambo, And H. K. Mukunza Comparison of five methods of malaria detection in the outpatient setting Am. J. Trop. Med. Hyg., 60(2), 1999, pp. 177–182
16. M. A. C. Dowling I & G. T. Shute2 A Comparative Study of Thick and Thin Blood Films in the Diagnosis of Scanty Malaria Parasitaemia Bull. Org. mond. Sant) 1966, 34, 249-267 Bull. World Health Org.J
17. D. Payne' Use and limitations of light microscopy for diagnosing malaria at the primary health care level Bulletin of the World Health Organization, (1988) 66 (5): 621-626
18. Fatima Shujatullah, Abida Malik, Haris M. Khan& Ashraf Malik Comparison of different diagnostic techniques in Plasmodium falciparum cerebral malaria J Vect Borne Dis 43, December 2006, pp. 186–190
19. Maline LM, Kyi MS, Chiodine PL. Accuracy of routine laboratory diagnosis of malaria in United Kingdom. J Clin Pathol 1998; 47: 740–2.
20. Lee MA, Awl T, Singh M. A comparison of antigendipstick assays with polymerase chain reaction and blood film examination in diagnosis of malaria. Am Acad Med Singapore 1999; 28(4): 498–501.
21. Bevinje Srinivas Kakkilaya, MD Rapid Diagnosis of Malaria laboratory medicine august 2003 number 8 volume 34 h602-608
22. M Metanat , B Sharifi-Mood Malaria vivax and Severe Thrombocytopenia in Iran Iranian J Parasitol: Vol. 5, No.3, 2010, pp. 69-70
23. Ammarah Jamal, Iqbal Ahmad Memon, Farheen Lateef. The association of Plasmodium Vivax malaria with thrombocytopenia in febrile children Pak Paed J Jun 2007;31(2):85-9.
24. Dilpreet Kaur, Vani Wasir, Sheffali Gulati, and Arvind Bagga Unusual Presentation of Plasmodium vivax Malaria with Severe thrombocytopenia and Acute Renal Failure Journal of Tropical Pediatrics Vol. 53, No. 3 Jan 2007 p 210-212]
25. Bhushan Katira & Ira Shah Thrombocytopenia in Plasmodium vivax infected children J Vect Borne Dis 43, September 2006, pp. 147–149]
26. Dhanpat K. Kochar , Ashish Das , Sanjay K. Kochar , Vishal Saxena , Parmendra Sirohi , Shilpi Garg , Abhishek Kochar , Mahesh P. Khatri , and Vikas Gupta Severe Plasmodium vivax Malaria: A Report on Serial Cases from Bikaner in Northwestern India Am. J. Trop. Med. Hyg., 80(2), 2009, pp. 194–198
27. Anupkumar R. Anvikar , Dinesh K. Singh , Ruchi Singh , Aditya P. Dash and Neena Valecha Vivax malaria presenting with cerebral malaria and convulsions Acta Parasitologica, 2010, 55(1), 96–98]
28. Lee SH, Looareesuwan S, Chan J, Wilairatana P, Vanijanonta S, Chong SM, Chong BH Plasma macrophage colony-stimulating factor and P-selectin levels in malaria-associated thrombocytopenia. Thromb Haemost. 1997 Feb;77(2):289-93
29. Park JW, Park SH, Yeom JS, Huh AJ, Cho YK, Ahn JY, Min GS, Song GY, Kim YA, Ahn SY, Woo SY, Lee BE, Ha EH, Han HS, Yoo K, Seoh JY. Serum cytokine profiles in patients with Plasmodium vivax malaria: a comparison between those who presented with and without thrombocytopenia Ann Trop Med Parasitol. 2003 Jun;97(4):339-44.
30. Ohtaka M, Ohyashiki K, Iwabuchi H, Iwabuchi A, Lin KY, Toyama K. A case of vivax malaria with thrombocytopenia suggesting immunological mechanisms Rinsho Ketsueki. 1993 Apr;34(4):490-2.
31. Erel O, Vural H, Aksoy N, Aslan G, Ulukanligil M. Oxidative stress of platelets and thrombocytopenia in patients with vivax malaria. Clin Biochem. 2001 Jun;34(4):341-4.
32. C. S. Scott2, D. Van Zyl1, E. Ho1, L. Ruivo2, B. Mendelow1, T. L. Coetzer1 Thrombocytopenia in patients with malaria: automated analysis of optical platelet counts and platelet clumps with the Cell Dyn CD4000 analyser Clinical & Laboratory Haematology Volume 24, Issue 5, pages 295–302, October 2002
33. National Antimalarial Programme (NAMP). Drug Policy. New Delhi : Directorate of National Antimalarial Programme. Ministry of Health andFamily Welfare, Govt. of India, 1996.]
34. WHO/UNICEF. Management of Childhood Illness. Chart Booklet. Geneva : World Health Organization, New York; United Nations Children Fund. 1995.