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COMPARISION OF RAPID DIAGNOSTIC TEST KIT VERSUS LIGHT MICROSCOPY FOR MALARIA DIAGNOSIS IN CHILDREN IN A TERTIARY CARE HOSPITAL IN CUTTACK CITY OF ODISHA,INDIA

Pathology

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KEYWORDS

Introduction:

Malaria is a common mosquito-borne disease and is one of the world's major public health problems contributing 243 million clinical cases and over a million deaths each year[1]. India leads the South East Asia region in terms of malaria cases[2].Odisha state with 3.8% of India's population accounted for 25% of total malaria cases,more than 40 % of Plasmodium falciparum malaria cases and nearly 20-30% of total malaria deaths in India[2]. It is characterized clinically by fever,rigor and chills and can be serious illness in children and can be fatal especially Plasmodium falciparum(Pf) malaria.Early and accurate diagnosis of malaria is essential for surveillance, proper management and prevention of death due to malaria. Overlap of malaria symptoms with other tropical diseases like typhoid fever, respiratory tract infections and viral infections impairs the specificity of presumptive diagnosis thereby encouraging indiscriminate use of antimalarials in endemic areas.World Health Organization recommends prompt diagnosis of malaria either by microscopic examination of blood smear or malaria rapid diagnostic test(RDT) in all clinically suspected malaria cases before administering anti-malarial drugs in their treatment[3].Diagnostic tests improves the overall management of febrile patients and may also help to reduce the emergence of drug resistance by reserving anti-malarial drugs for those who actually have malaria.The rapid diagnostic test (RDT) is a quick and easy test when compared with microscopic examination of blood smear which takes more time and require good quality microscopy and competent technical expertise.Malaria rapid diagnostic tests having high sensitivity and negative predictive value particularly for plasmodium falciparum malaria have the potential to significantly improve management of malaria infections especially in children where Pf malaria could have been fatal.

The basis of the study was:

(1) To evaluate the clinical usefulness of examination of Rapid Diagnostic Test(RDT) for malaria in children and
(2) To compare its performance to blood smear microscopy.

Materials and Methods:

The present studty was cross-sectional and conducted in Sardar Vallabhbhai Patel Post Graduate Institute of Paediatrics(Sishu Bhawan),Cuttack ,in the state of Odisha.Partcipants were all children with pyrexia or with present history of fever since lst one week with clinical suspicion of malaria who attended the Outpatient Department of Sardar Vallabhbhai Patel Post Graduate Institute of Paediatrics (Sishu Bhawan),Cuttack,during the period 1st September 2015 to 31st August 2016.

Inclusion criteria: Patients of paediatric age (upto 14 years) both male and female who were having clinical suspicion of malaria as evidenced by fever(temp 99 F or more) associated with rigor and chill or having present history of such fever within one week.

Exclusion criteria: Complete absence of malaria symptoms,those children who had already taken or started antimalarial drugs ,those who had fever with obvious cause of bacterial or viral infections, in whom fever was associated with cough and breathlessness or symptoms of Urinary Tract Infection and when there was unwillingness by parents to participate.

A total of 1126 children with fever with clinically suspected malaria were investigated. Biodemographic information obtained include age,gender,presenting symptoms,other symptoms,duration of fever and history of treatment received,all patients were examined and clinical findings were documented, temperature was measured in axilla using clinical thermometer.All the recruited subjects were investigated for microscopic examination of malaria parasite in blood smear using Leishman stain and malaria rapid diagnostic test(RDT) was performed using SD Bioline Malaria Antigen P.f/P.v Rapid test.

The above rapid diagnostic test is a device that detects malaria antigen in a small amount of blood, usually 5 µL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen pLDH/HRP2 , impregnated on a test strip. The result, usually a colored test line, is obtained in 5–20 min. RDTs require no much of capital investment or electricity, are simple to perform, and are easy to interpret.

Sample collection,preparation,staining and associated work were performed by trained and experienced technicians working in the institution following standard operating procedure.

Blood collection and analysis of RDT and microscopic examination: Under all aseptic measures one ml of blood was collected from antecubital vein of febrile children in to EDTA vials.Rapid diagnostic test(RDT) for malaria was performed on about 5 micro liter of blood using SD Bioline Malaria Antigen P.f/P.v Rapid test kit which is an one step,rapid,qualitative and differential test for the detection of HRP-II(Histidine-rich protein II) specific to Plasmodium falciparum and pLDH (Plasmodium lactate dehydrogenase) specific to Plasmodium vivax in human blood sample.

The test was conducted following the detailed procedure of the test and result interpretation was done as Negative,Positive (P.f./P.v/Mixed) or Invalid according to instruction and information given by the manufacturer of the kit. A drop of blood was used for preparing a thick and a thin smear which was dried and stained with leishman's stain for 10 minutes,washed with distilled water ,dried and examined carefully under oil-immersion lens of microscope and atleast 200 fields were examined before reporting a smear as negative .Because of the fixed monolayer of RBC available in thin smear the morphological identification of the parasite to the species level was much easier and provided greater specificity than the thick-smear examination. Thus thin blood film was preferred for exaimation of the parasite because the organisms were easier to detect and could be differentiated in to their species. All the slides were examined by two independent pathologists and all positive cases were confirmed by both of them.

- The results of both RDT and blood smear microscopy were compiled,statistically analysed and P value <0.05 was taken as significant..The diagnostic performance of malaria rapid diagnostic tests(RDT) was compared to blood smear microscopy in febrile children in Sishu Bhawan,Cuttack in the state of Odisha.

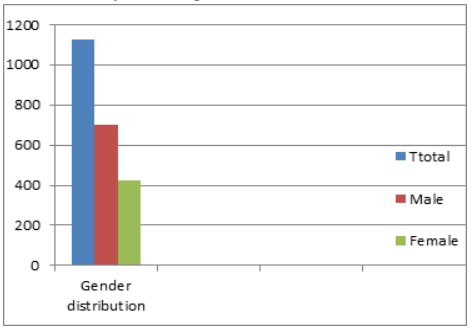
Results

A total of 92215 children attended Out Patient Department of Padiatric medicine of Sardar Vallabhbhai Patel Post Graduate Institute of Paediatrics(Sishu Bhawan), Cuttack, during the study period of one year.Out of which 4320(4.68%) children were having Pyrexia or history of fever during last one week.From these fever cases by thorough clinical history and examination only 1126(26.1%) children were identified to be clinically suspected malaria cases who constitute the study group(Table 1)

Period of study	Total OPD cases	No.of Fever cases	%	No.of cases with suspicion of malaria	%	Positive In RDT	%	Positive in microscopy	%
1/9/2015 to 31/8/2016	92215	4320	4.7	1126	26	97	8.6	81	7.2

Table:1 :Showing incidence of Fever , Suspected malaria and test positivity

Out of 1126 cases examined; 702 (62.3%) were male and 424(37.7%) were female ,674(59.9%)were below 10 years age and 452(40.1%) were between 10-14 years of age.



All 1126 fever cases included in the study were subjected to both RDT and blood smear microscopy and the results were compiled and compared; 97(8.6%) tested positive by rapid diagnostic test and 81 (7.2%) by blood smear examination. In this study a total number of 16 cases(13Pv,3Pf) were found who were positive by RDT but no malaria parasite could be detected in their blood smear microscopy which showed that 16.5% of RDT positive cases could have been missed of malaria diagnosis if relied on only blood smear microscopy.

Type of Test	Total no of test done	Total positive	%	Pf	%	Pv	%	Mixed	%
RDT	1126	97	8.6	78	80.4	13	13.4	6	6.2
Microscopy	1126	81	7.2	68	83.9	9	11.1	4	4.9

Table :3: Comparision of RDT with Microscopy

Out of 97 cases who were positive by RDT, Pf,Pv,and mixed infection constituted 78(80.4%) , 13(13.4%) and 6(6.2%) cases and out of 81 positive cases detected by microscopy the species were Pf 68(84%),Pv 9(11%) and Mixed 4(5%) respectively(chart below).

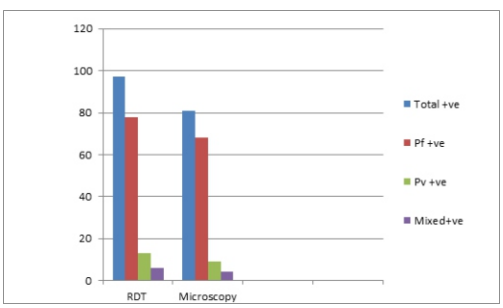


Chart showing results of RDT and Microscopic examination of Blood-smear The sensitivity of the rapid diagnostic test for malaria was 99.8%, specificity was 98.6%,with P value of 0.060 indicates that there is no significant difference between diagnostic performance of RDT and blood smear microscopic examination[4]. For detection of Plasmodium falciparum the sensitivity of rapid diagnostic test was 100% and specificity was 98.6% and for pl.vivax the sensivity was 98%.

Most new rapid tests for malaria diagnosis incorporates immunoch romatographic capture procedures, with conjugated monoclonal antibodies providing the indicator of infection. Preferred targeted antigens are those which are abundant in all asexual and sexual stages of the parasite and are currently centered on detection of HRP-2 from Plasmodium falciparum and parasite-specific lactate dehydrogenase from plssmodium vivax species, which is the basis of RDT. Clinical studies allowed effective comparisons between RDT and microscopy, and the reality of nonmicroscopic diagnoses of malaria by RDT is considered an easy to perform test with acceptable sensitivity, specificity and reliability.

DISCUSSION

In most endemic countries malaria diagnosis depends mainly on clinical evidence and in some cases thick and thin smear microscopy and rapid diagnostic technique (RDT) may be used for laboratory confirmation. Microscopy is regarded as the gold standard for malaria diagnosis with a threshold sensitivity of 5 to 50 parasite/µL (depending on the microscopist expertise) [5], But the major constraints of microscopy include the requirement of considerable technical expertise and the fact that it is time- consuming for optimal blood film preparation, examination and interpretation [6]. Therefore the shortcommings of microscopic diagnosis of malaria has long been recognized in practice and involves multiple factors, including training and skills, slide preparation techniques, workload, condition of the microscope, and quality of essential laboratory supplies. Even among local laboratories with similar equipment and equal training and among reputed experts, abilities vary significantly[11,12].

RDT, an immunochromatographic capture procedure was developed to improve the timeless sensitivity, and objectivity of malaria diagno sis through less reliance on microscopy [2].In children in high-transmission areas clinical diagnosis and RDTdetermine the treatment decision on malaria[9,10].In our study we have used SD Bioline Malaria Antigen P.f/P.v Rapid test which is a device that detects malaria antigen in a small amount of blood, usually 5 µL, by immunoc hromatographic assay with monoclonal antibodies directed against the target parasite antigen pLDH/HRP2 , impregnated on a test strip. The result, usually a colored test line, is obtained in 5–20 min. Histidine-Rich Protein 2 (HRP-2) is the most common malaria antigen targeted and is specific for P. falciparum. Parasite lactate dehydrogenase (pLDH) enzymes are the other major group of targeted antigens targeted and is specific for P.vivax.However, plasmodial gametocytes also produce pLDH and so a pLDH test may remain positive despite clearance of the asexual parasite forms. Persistent HRP-2, on the other hand, could be an advantage in detecting low-level, fluctuating parasitemia in chronic malaria[13,14].

From the 4320 febrile children who attended the hospital , only 1126(26.1%) children were identified to be clinically suspected malaria cases which correlates with the study of Rajagopalan et al.[27] The burden of malaria was generally higher in men than women in all age groups. In the present study, it was shown that overall; malaria prevalence was higher among males (62.3%) than females(37.7%) The significantly ($P < 0.05$) higher malaria positivity rate recorded in male-children compared to that in females could be due to the dressing pattern of females who fully cover their bodies and stay indoors, while male-children are mostly involved in outdoor game and activities and are likely to get more mosquito bites compared to females.This finding correlates the finding of Das NG et al [20]and that of Isa abdulkadir et al[28] .

Age-specific analysis indicated that children (up to ten years) had higher prevalence of malaria compared to children of 10-14 years of age.About 60% children were under 10 years of age and rest 40% were 10 to 14 years of age. Similar observation was made in Assam[24], Arunachal Pradesh[25] and Rajasthan[26].

Out of the four human malaria parasites, two parasite species(Pf and

Pv) were recorded in the current study. Plasmodium falciparum was found to be predominant malaria parasite which was also known to be prevalent in the same proportion in other districts of Odisha[21].

Similar to the findings of the present study, in Sundargarh district of Odisha State, P. falciparum accounted for 85.0% of the total malaria cases[22]. In recent past, chloroquine-resistant P. falciparum strain was detected with increasing frequency in many districts of Odisha State as well as in the country[23].

To be a useful diagnostic, RDTs must achieve greater than 95% sensitivity [8].The Sensitivity for P.

falciparum is excellent (> 95%) in some studies and poorer (80%+) in others[15,16,17]. In our study it was found to be 98.8% hence excellent and useful unlike the finding of Isa abdulkadir,H A Rufai et al [28]

In spite of over 100 published RDT trial reports, comparative assessment is difficult because (1) trials do not share common guidelines; (2) clinical and epidemiologic characteristics of the study populations, especially the parasitemia level vary; (3) reference standards are different; even among those using same stain microscopist skills vary; and (4) products of different lots may differ in quality or be damaged by extreme temperature or humidity during transportation and storage.In areas where good microscopy has failed or never reached, RDTs are recommended, in situations exceeding microscopy capability, such as in an outbreak RDTs are also recommended and the scope of RDT applications will expand however currently RDTs are not intended to replace microscopy.The use of RDTs at peripheral levels such as by health workers, in informal health sectors and for self-diagnosis/self-treatment is a challenge[18,19].

Conclusion:

The rapid diagnostic test (RDT) is a quick and easy test which should be encouraged for screening and diagnosis using a protocol such that febrile children with positive RDT results are confirmed as having malaria while those with negative result are further evaluated using microscopy.RDT is a valuable complement to microscopy because it helps expand the coverage of parasite-based diagnosis to the periphery and minimize exclusively clinical diagnosis. International health agencies and the scientific community engaged in epidemiology, drug, and vaccine work need to urgently put forth an effort to improve the global capacity to diagnose malaria.In this context,Rapid diagnostic test (RDT) is cost-effective and offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available right away due to any reason.

Successful implementation of RDTs requires integrated planning and effort for proper procurement and correct use of the kit following a new local-level algorithms for actions to be taken based on RDT results even if microscopic diagnosis of malaria is not available.

The limitation of the study: RDT may not be able to detect some infections with lower numbers of malaria parasites in the patient's blood and the less common species of malaria like P. ovale and P. malariae, in the malaria-endemic world.

Declaration:Ethics approval and consent to participate:Consent obtained from parents of children for participation and the study was approved by the institutional ethical committee. Mohapatra C S designed and implemented the study. Bramha R C and Mohanty S supervised all the laboratories activities.

All authors are actively involved in the study, read ,provided critical comments,with substantial contribution in acquisition ,analysis and interpretation of data and approved the final manuscript.

Competing interest:None

Source of funding-Nil

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