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Reliability of Rapid Diagnostic test in Diagnosis of Malaria

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ABSTRACT

Aim: To determine the reliability of the Rapid diagnostic test compared to the traditional peripheral smear for the diagnosis of Malaria. To assess whetherMalaria Ag Pf/Pan test is an effective and sensitive tool in the diagnosis of Malaria. Method: Cross sectional diagnostic study was done in Department of Pediatrics, Government Royapettah Hospital. Children 6 months to 12 years are admitted with Fever with chills and rigor with splenomegaly. Intervention: Malaria Ag Pf/Pan card test was used as a rapid diagnostic test. A peripheral smear was prepared at the same time and Leishman stain was used to stain the smear and examined on the same day. Results: Malaria Ag Pf/Pan test is an effective and sensitive tool in the diagnosis of Malaria. It has a very high sensitivity and specificity of 100% for detection of plasmodium falciparum. This test has a high sensitivity of 100% and a specificity of 86.6% for detection of Plasmodium vivax. It has high Positive predictive values of 94.1% and a very high negative predictive value of 100% in diagnosing malaria.Conclusion: Rapid diagnostic test (Malaria Ag Pf/Pan) is a simple and effective test which can be done at bed side for diagnosing malaria.

KEYWORDS : Rapid Diagnostic Test, Malaria, Leishman stain

Introduction

Malaria is of overwhelming importance in the developing world with an estimated 300 million cases occurring every year and more than 1 million deaths each year.[1] Most of the deaths due to malaria occur in infants and young children. All clinically suspected cases of malaria were treated with Chloroquine in the past. This has led to the emergence of Chloroquine resistant P. falciparum. Infection due to P. falciparum is Severe and hence, early detection is essential for starting appropriate therapy. In developing countries such as India, the case fatality rate due to cerebral malaria is 30-40%.[2] National Health Policy (2002) and Millennium Development goals aim at reduction of mortality due to malaria by 50% by the year 2010. Although microscopy remains the gold standard for diagnosing malarial infections, various immune-chromatographic tests which detect malarial antigens have been developed for rapid diagnosis of malaria.[3]It is useful not only in diagnosing malaria, but also in confirming cure after treatment, because the test will detect only live parasites, and so will be negative if the parasites have been killed by treatment.[4.] An easy, reliable and rapid diagnostic test with high sensitivity and negative predictive value would certainly be clinically useful.[5]

Materials and Methods:

The Cross sectional diagnostic study was conducted in Department of Pediatrics at Government Royapettah hospital (Kilpauk Medical College) from January 2011 to June 2012. Ethics committee approval and Informed consent from the children's parent were obtained. Children admitted in our ward presenting with Fever with chills and rigor with splenomegaly in the age group of 6months to 12 years were included. Children diagnosed and treated with anti-malarial drugs were excluded**.** Malaria Ag Pf/Pan Card test was used as a rapid diagnostic test. After obtaining an informed consent, 5 microliter of blood was obtained using a sample applicator pipette which was delivered into the sample well. 4 drops of buffering solution was delivered into the developer well. The buffering solution was used to wash away the hemoglobin and leave the malarial antigen to bind with the corresponding monoclonal antibodies which are pre-coated in the card. The results are read in 5-10 minutes. A peripheral smear was prepared at the same time and Leishman stain was used to stain the smear and examined on the same day by an experienced microbiologist without reference to the RDT result. Results of rapid diagnostic test were compared with peripheral smear results.

Results:

Table 1 Comparison of Conventional Microscopy Verus Rapid Diagnostic Test In the Diagnosis of Malaria

Positive		PHERIPHERAL SMEAR		Total
		Negative		
RDT	Positive	273	17	290
	Negative	0	110	110

Our study has shown that rapid diagnostic card test had an excellent sensitivity of 100% and a specificity of around 86.6% as compared to microscopy. Rapid Diagnostic card test had a positive predictive value of around 94.1% and a negative predictive value of around 100% as compared to microscopy. False positive cases were 17, which constitutes around 13.4%. Likelihood ratio is 7.5%. Accuracy of Rapid diagnostic test is 95.75%. (P value = 0.002). (Table 1)

Table 2 Comparison of Rate of Detection of P.Vivax by Conventional Microscopy Versus Rapid Diagnostic Test

		P.VIVAX		Total
	PHERIPHERAL SMEAR			
	Positive	Negative		
P.VIVAX-RDT	Positive	235	17	290
	Negative	0	148	110

Our study has shown that rapid diagnostic card test had an excellent sensitivity of 100% and a specificity of around 89.69% for P.vivax as compared to microscopy. Rapid Diagnostic card test had a positive predictive value of around 93.2% and a negative predictive value of around 100% for P.vivax as compared to microscopy. False positive cases were 17, which constitutes around 10.3%. Accuracy of the rapid diagnostic test in detecting P.vivax is 95.75% (P value <0.0001) (Table 2).

Table 3 Comparison of Rate Of Detection of P .falciparum By Conventional Microscopy Versus Rapid Diagnostic Test

		P.FALCIPARUM		Total
	PHERIPHERAL SMEAR			
	Positive	Negative		
P.FALCIPARUM	Positive	38	0	38
	Negative	0	362	361

Our study has shown that rapid diagnostic card test had an excellent sensitivity of 100% and a specificity of 100% for P. falciparum as compared to microscopy. Rapid Diagnostic card test had a positive predictive value of 100% and a negative predictive value of 100% for P. falciparum as compared to microscopy. Accuracy of the rapid diagnostic test in detecting P. falciparum is 100% (P value <0.0001) (Table 3).

Table 4 Performance of Rapid Diagnostic Card Test In Comparison With Conventional Microscopy in Diagnosis of Malaria

Sensitivity	100.0
Specificity	86.6
PPV	94.1
NPV	100.0
False positive	13.4
False negative	0.0
Likelihood ratio(Positive)	7.5
Accuracy	95.75

Discussion

The purpose of this study is to assess Reliability of Rapid diagnostic test in diagnosis of malaria. In spite of recent advances in the rapid diagnosis of malaria, microscopic examination of the peripheral blood smear remains the gold standard for diagnosis of malaria. However this approach requires an organized health care system, infrastructure, with functioning microscopes, trained technicians, reagents, supervision, and quality control.[6] Rapid antigen detection of lactate dehydrogenate (p LDH) and Histidine-rich Protein II (Pf HRPII) released from the parasitized RBCs offers a simple and efficient method for detection of malarial cases by even untrained personnel within 5-10 minutes. Early detection of malarial parasites goes a long way in preventing further morbidity and development of the dreaded complications of malaria.[7] An easy, reliable and rapid diagnostic test with high sensitivity and negative predictive value would certainly be clinically useful.[8] Malaria Combo (Pf HRPII/P LDH) test contains a card, which is pre-coated with two monoclonal antibodies represented as two separate lines. One of the monoclonal antibodies is pan specific to lactate dehydrogenase (pLDH) of plasmodium species (P. falciparum, vivax, malariae, ovale) and the other line consists of a monoclonal antibody specific to histidine rich protein II (HRPII) of the P. falciparum species. The conjugate pad dispenses monoclonal antibodies, which are pan specific to LDH and P. falciparum specific to HRPII. The blood sample is measured in a calibrated dropper capable of delivering a quantity of 5 microliter accurately into sample well followed by two drops of the assay buffer (60 microliter) into developer well. The test card has one control line that indicates the validity of the test procedure and its working condition. Control and test lines appeared within 5-10 minutes in a reading window. The interpretation of the test is as described: PLASMODIUM FALCIPARUM POSITIVE REACTION: The presence of three bands (control, test line 2, test line1) or two bands (control, test line 1) indicates positive results for P. falciparum or P. falciparum Plus other non-falciparum species). PLASMODIUM VIVAX OR OTHER PLASMODIUM SPECIES POSITIVE REACTION: The presence of two bands (control, test line 2) indicates a positive result for non-Falciparum malaria. NEGATIVE REACTION: The presence of only one band in the control area indicates a negative results.[9] RDT Target antigen: Histidine -rich protein II (HRP-II): It is a water soluble protein produced by trophozoites and young gametocytes of P. falciparum. Parasite lactate dehydrogenase (p LDH): Enzyme is produced by both sexual and asexual stages of all malarial parasites. They can distinguish P. falciparum from non-falciparum species, but cannot distinguish between P. vivax, P. ovale and P. malariae. RDTs: Test performance: RDTs may detect all four Plasmodium species that infect humans, or may detect only falciparum depending on the antigens on which they are based. Present RDTs fail to differentiate between P. vivax, P. ovale, and P. malariae. RDTs generally achieve a sensitivity of more than90% in the detection of P. falciparum at densities above 100 parasites per micro litre of blood. However with low Parasitemia, sensitivity decreases markedly. HRP-II tests can remain positive for 7-14 days following chemotherapy in a proportion of individuals. RDTs: Advantages: RDTs are easy to perform and interpretation is simple enough to be grasped even by an untrained person. RDTs do not require special equipment or training. Technique can be learnt within a few hours. Since, RDTs detect circulating antigens; they are capable of detecting P. falciparum infection which is sequestered in the deep vascular compartment. RDTs can be stored under ambient conditions. RDTs Disadvantages: RDTs targeting HRP-II of P. falciparum could give false positive results for a fortnight beyond parasite clearance as confirmed by microscopy. Kits that detect both P. falciparum and non-falciparum species are unable to differentiate between P.vivax, P.ovale and P.Malariae. RDTs are not quantitative, hence are useless for prognostication. RDTs are more expensive than microscopy. False positive results can occur due to cross reaction with auto-antibodies such as Rheumatoid factor. False negative results can occur due to pro-zone phenomenon at a high level of antignemia.[10] The cost of Malaria Ag Pf/Pan test is high when compared to microscopy which may prevent its routine use in practice. But it is a useful adjunct in diagnosing clinically suspected cases of malaria so as to initiate treatment where expertise for peripheral smear examination is not available and in hospitals where work load is high which may delay the results and it is easier to use in routine office practice.[11] Moreover there is no role for empirical treatment of malaria with chloroquine. Prompt parasitological confirmation by microscopy or alternatively by RDTs is recommended in all patients suspected of malaria before treatment is started.[12] Treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible. WHO also recommends the use of Rapid diagnostic test in the diagnosis of malaria.[13] WHO theme for the year 2011 was "NO ACTION TODAY, NO CURE TOMORROW".

Conclusion

This study concludes that Rapid diagnostic test (Malaria Ag Pf/Pan) is a simple and effective test which can be done at bed side for diagnosing malaria. It has a very high sensitivity and specificity of 100% for detection of plasmodium falciparum. This test has a high sensitivity of 100% and a specificity of 86.6% for detection of Plasmodium vivax. It has a high Positive predictive value of 94.1% and a very high negative predictive value of 100% in diagnosing malaria. Moreover RDTs does not require highly skilled personnel to perform or interpret results. Though the cost of the test may prevent its routine use, it is a better option in diagnosing malaria rapidly and avoiding delay in initiating treatment. Compared to peripheral smear examination, it is easier to use in routine office practice.

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