EVALUATION OF TWO IMMUNO CHROMATPGRAPHIC BASED KITS FOR RAPID DIAGNOSIS OF MALARIA

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ABSTRACT

Objective: *To evaluate the performance of two different immuno chromatographic based devices, developed for the rapid diagnosis of malaria without the aid of a microscope.*

Material and Methods: *This prospective study was carried out in Pathology Department PGMI/LRH, Peshawar from May to December 2003. A total of 50 blood samples were collected, 41 from patients, who were smear positive on conventional microscopy and 09 smear negative healthy individuals. Thick and thin smears were stained with Giemsa's stain. Tests were performed on Now ICT and OptiMAL devices according to the instructions provided by the manufacturers.*

Results: *On microscopy there were 16 positive cases of P. vivax and 25 positive cases of P. falciparum. ICT and optimal demonstrated 14 and 12 cases positive for P. vivax respectively while 25 out of 25 cases were turned out to be positive for P. falciparum on both the devices showing 100% sensitivity and specificity for P.falciparum and 75 to 87.5% sensitivity and 100% specificity for P.vivax.*

Conclusion: *These devices were found highly sensitive and specific for the detection of malarial parasites in peripheral blood and can be used as a first line diagnostic tool.*

Key words: *Histidin Rich Protein 2 (HRP2), Plasmodium Specific Lactate Dehydrogenase (pLDH) Immuno-Capture Devices.*

INTRODUCTION

Malaria is considered as one of the most widely spread and prevalent disease of man kind. It is the fifth largest killer out of all the infectious diseases of the world. It strikes young children, non immune adults and women during their first pregnancies.[1] It has had the resurgence in many parts of the globe due to insecticides and drug résistance, social instability and non availability of antimalarial vaccine. [2-3] Early diagnosis and prompt adequate treatment for malaria are paramount in the prevention of mortality. Microscopy of thick and thin blood film is still the method of choice for malaria diagnosis but it requires experience and the presence of considerable number of parasite in the peripheral blood.[4] Therefore various immuno capture devices have been introduced which were proved to be highly sensitive and specific for on site confirmation of malaria.[5-16] Plasmodium synthesize several proteins which contain large amount of Histidine referred to as Histidine Rich Protein (HRP), One of these is HRP2.It is a water soluble protein, releases from infected erythrocytes. The other marker protein is plasmodium specific lactate dehydrogenase (pLDH) synthesized by all the stages of intraerythrocytic parasites of malaria.

Binax (USA) has introduced *NOW* ICT malaria kit which is a rapid invitro immunochromatographic test for the detection of circulating Histidine Rich Proteins. Flow Inc has developed the OptiMAL malaria device which can detect Plasmodium Lactate Dehydrogenase.

As an accurate and rapid diagnosis of malaria is essential for effective and prompt treatment to over come the morbidity and mortality therefore an evaluation study was designed to assess the sensitivity and specificity of these newly developed systems by comparing them to microscopic examination.

MATERIAL AND METHODS

This study was conducted at Pathology

Test result of ICT malaria and OptiMAL compared to Microscopy

Species	Microscopy	NOW ICT	OptiMAL
P.Vivax (+ve)	16	14	12
P.Falciparum (+ve)	25	25	25
MP Negative	09	09	09
Total	50	48	46

Table 1

Department of Post Graduate Medical Institute Lady Reading Hospital Peshawar. A total of fifty blood samples were collected 41 from patients with clinical diagnosis of malaria who were smear positive and 9 healthy individuals having no clinical symptoms and were smear negative for malarial parasite. 2ml of venous blood was drawn into EDTA coated tubes. Thick and thin smears of blood were made and stained with 2% Giemsa's stain in phosphate buffered saline(pH 7.2) and examined under the microscope for the plasmodium. Slides were examined and reported independently by two experts.

ICT/OptiMAL tests were performed "blind" without prior knowledge of the microscopic results. Blood samples were made according to the brochure provided by the manufacturers.

RESULTS

50 blood samples were tested for the presence of malaria by NOW ICT and OptiMAL devices and the result were compared to the finding obtained from microscopic examination (Table 1-2).

On microscopy there were 16 positive cases of P. vivax and 25 positive cases of P. falciparum. ICT and optimal demonstrated 14 and 12 cases positive for P. vivax respectively while 25 out of 25 cases were turned out to be positive for P. falciparum on both the devices showing 100% sensitivity and specificity for P.falciparum and 75 to 87.5% sensitivity and 100% specificity for P.vivax.

DISCUSSION

This comparative study of two malarial antigen capture devices (ICT P.f/P.v and OptiMAL) with conventional microscopy for the diagnosis of malaria appears to be approximately equivalent in sensitivity and specificity to those extensively studied in a number of different countries.

Malaria is seasonal and both *P.vivax* and *P.falciparum* are common while *P.falciparum* is mainly prevalent during monsoon and post monsoon period (July to January). Malaria during dry season (February to June) is mainly due to *P.vivax*.[9-10]

As this study was conducted from May and December therefore both *P.vivax and P.falciparum* cases has been observed.

Evaluation so far carried out have shown ICT and OptiMAL to be sensitive and specific tests for the diagnosis of malaria. The results of this study demonstrates that ICT was 87.5% and OptiMAL 75% sensitive to P.vivax while the specificity of both the devices were 100% which is comparable to the study conducted by Rahim and Jamal in 2002[7]. Their ICT tests had shown 81% sensitivity and 100% specificity for P.vivax. Palmer in 1998[5] had demonstrated 94% sensitivity and 100% specificity of OptiMAL test for P.vivax. Singh etal in 2000[10] carried out tests in remote villages of India and found ICT malaria to have a

Sensitivity and Specificity of the NOW ICT and OptiMAL versus microscopy.

P.Vivax	NOW ICT	OptiMAL
Sensitivity	87.5%	75%
Specificity	100%	100%
P.Falciparum	------	------
Sensitivity	100%	100%
Specificity	100%	100%

Table 2

sensitivity of 100% and a specificity of 84.5%. Both the devices ICT and OptiMAL has shown 100% sensitivity and specificity for P.falciparum which is comparable to the study conducted by Hashmi and Haider in 1998[17]. As observed by Garcia etal in 1996 that ICT test could detect malaria when the density of parasite exceeds 80/l. In the present study two cases were found positive for P.vivax on microscopy but both the devices were non reactive on the first day of the symptoms but became reactive on the second day showing poor sensitivity of the devices at the lower level of parasiteamia. The WHO global malaria strategy in 1992[19] recommends rapid diagnosis of malaria at the primary health care level so that effective treatment can be started quickly to reduce morbidity and mortality besides the financial saving from unnecessary treatment. The use of non microscopical malaria tests is of value in the early investigation and management of malaria. The rapid tests are also of value in the diagnosis of severe and complicated falciparum malaria when microscopy is not possible and in diagnosing malaria in those who have taken antimalarials. Per test cost of the ICT/OptiMAL device is more as compare to the cost of microscopy to diagnose malaria but these are exceptionally easy to perform therefore they can serve as a useful addition to microscopy in tertiary health care units and first line diagnostic tool in primary health care units.

CONCLUSION

Malaria is considered as one of the most widely spread and prevalent disease of mankind. P. falciparum is the main cause of severe clinical malaria and mortality. It strikes young children, non-immune adults and women during their first pregnancies.

Microscopy of thick and thin blood film is still the method of choice for malaria diagnosis but it requires experience and is time consuming and therefore does not suit either to the rural settings where the laboratory staff lacks the proper training and experience or to the urban hospital laboratories where the heavy workload is the main cause for missing of the positive cases of malaria.

Various immuno capture diagnostic assays have been proved to be highly specific and sensitive for on site confirmation of malaria.

This study was conduced to evaluate the performance of two rapid non microscopic assays. Only two cases of P. Vivax were not detected by *NOW* ICT and four cases were missed by OptiMAL while no case of P. Falciparum was missed by the either device.

Compare to microscopy the performance of both the assay to detect the malaria was found satisfactory.

As these tests are easy to perform therefore they can serve as a useful addition to microscopy in main laboratories and first line diagnostic tool in peripheral laboratories.

Acknowledgements

I acknowledge the efforts of Mr. Waleed student of Bioinformatics COMSATS Islamabad for giving his valuable time in the preparation of this manuscript. I also acknowledge the efforts of Mr. Niaz Mohammad Chief Lab. Technician Govt. LRH, Peshawar and Mr. Syed Zewar Shah Sr. Lab. Assistant Govt. LRH, Peshawar for their help in the preparation of slides. I am also grateful to Mr. Shah Iran Computer Operator Govt. LRH, Peshawar.

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