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COMPARISON OF IMMUNOCHROMATOGRAPHY AND MICROSCOPIC FILM METHOD FOR THE DIAGNOSIS OF MALARIA IN LIBERIA

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ABSTRACT

Objective: To compare immunochromatography and microscopic film method for the diagnosis of malaria in patients reporting to United Nations deployed hospital in Liberia.

Study Design: Diagnostic accuracy study.

Place and Duration of Study: Departments of Pathology and Medicine, United Nations deployed hospital in Liberia, West Africa, from Feb 2015 to Dec 2016.

Methodology: A total of 250 febrile patients of all ages and both genders reporting to United Nations deployed hospital in Liberia, with typical clinical features of malaria and advised for workup of malarial parasite were included. The febrile patients with no typical clinical features of malaria and negative malarial parasite both on immunochromatography and microscopic methods were excluded. Pretreatment whole blood in ethylenediamine tetraacetic acid was collected for testing malarial parasite on immunochromatography and comparing with gold standard microscopic film method. On 4th day of treatment with tablet Artemether + Lumefantrine 40/240 mg in double strength (DS), whole blood in ethylenediamine tetraacetic acid was tested again for malarial parasite on immunochromatography and microscopic film method.

Results: In total of 250 patients, 150 were found negative for malarial parasite both on immunochromatography and film method. Out of 100 cases, found positive for malarial parasite on any of the methods, 75% were males and 25% were females with mean age of 38 + 5 years. The type of malarial parasites found in all positive cases was plasmodium falciparum. Pre-treatment malarial parasite was found positive on microscopic film in 95% cases and on immunochromatography in 88% cases. Mean malarial parasite index was 0.49% ranging from 0 to 3.5%. Post-treatment malarial parasite was found positive on immunochromatography in 88% cases and in 14% cases on malarial parasite film. Post treatment mean malarial parasite index was 0.04% ranging from 0 to 1.8%. Pretreatment malarial parasite immunochromatography sensitivity was 87% and specificity was 97%.

Conclusion: Immunochromatography provides rapid, sensitive, user friendly and practical alternative to slide microscopy for the diagnosis of malaria especially in remote areas, despite the highlighted shortcomings of false-negative and false-positive results.

Keywords: Malarial Parasite, Malarial parasite index, Immunochromatography.

INTRODUCTION

Malaria is a common, life-threatening infection in endemic tropical and subtropical areas. According to WHO report on malaria 2015 an estimated 214 million malaria cases occurred globally with largest number (88%) from Africa and plasmodium falciparum causing more than 90% cases in this region[1]. A rapid and accurate diagnosis is most important for timely and effective treatment, especially for the potentially fatal cases of plasmodium falciparum infection. Currently, the vast majority of malaria cases are detected by light microscopy of stained blood smears which remains the gold standard for malaria diagnosis[2]. Microscopic examination is time consuming and requires an experienced person particularly at low levels of parasitemia and interpretation of mixed infection.

Several simple and non-microscopic rapid diagnostic tests (RDTs) have been developed to facilitate malarial diagnosis[3]. These immunodiagnostic tests are easy to use and can produce fast results, but they usually have lower sensitivity and specificity than conventional microscopic examination[4]. On the other hand, use of molecu-

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lar biology for the diagnosis of malaria has proved to be highly sensitive but is not widely available and the protocol is more complex and needs better trained technicians[5]. Malaria RDTs/ Immunochromatography (ICT) are increasingly used for diagnosis of malaria, particularly in remote tropical areas where good microscopy-based diagnosis is usually not available[6]. To be useful in malaria diagnosis, ICT must achieve greater than 95% sensitivity.

Liberia is a hyperendemic area for malaria and more than 90% cases are caused by plasmodium falciparum. Both health care facilities as well as expertise are sparse and the purpose of our study is to evaluate the usefulness of ICT method as compared to gold standard microscopic method in this area to determine the sensitivity and specificity of ICT method for rapid and accurate diagnosis of malaria and to save precious lives.

METHODOLOGY

This cross-sectional study was conducted in the departments of pathology and medicine, UN deployed hospital in Liberia, West Africa from February 2015 to December 2016 after approval of the institutional ethics review board (IERB #2). Sample size was calculated using a standard formula of WHO sample size calculator. Briefly, using the prevalence data 88% in Africa[1], precision 0.05 (5%) and statistical power 80%, the sample size was 163 subjects. A total of 250 febrile patients of all ages and both gender with typical clinical features of malaria and advised for workup of malarial parasite were included consecutively. The febrile patients with no typical clinical features of malaria and negative malarial parasite (MP) both on ICT and microscopic methods were excluded.

Two hundred fifty samples were consecutively collected from all the patients after their informed consent for the workup of malaria. History of illness, findings of physical examination, demographic data and baseline routine investigations were carried out at the start of the study. All samples were collected in vacutainer tubes (BD, NJ USA). Pretreatment whole blood in EDTA was collected for testing MP by ICT [anti histidine rich protein-II (anti-HRP-II) plasmodium falciparum specific/p.f and anti aldolase antibodies/pan line for all malarial parasite antigens] and microscopic film method including MP index. Rapid diagnostic test is a device that detects malaria antigen in a small amount of blood, usually 5-15 µL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. The result, usually a colored test line, is obtained in 5–20 min. In microscopic film method including MP index, Leishman stain was used and all the slides were seen and verified by pathologist. All cases of malaria were treated with tablet Artem DS (Artemether + Lumefantrine) 40/240 mg in double strength (DS), two tablets twice daily for three days. On fourth day of treatment, whole blood in EDTA was again collected from all the cases and tested for MP by ICT and microscopic film method including MP index.

Statistical analysis of all the data was entered in statistical package for social sciences version 17 (SPSS Inc, Chicago, IL, USA). Mean and ranges were calculated for quantitative variables like age and MP index. Frequencies and percentages were calculated for qualitative variables like gender and positive malarial parasite by ICT and microscopic film methods. ROC curve was drawn to see sensitivity and specificity of ICT in comparison of MP film on microscopy.

RESULTS

In total of 250 patients, one hundred and fifty were found negative for MP both on ICT and film method. Out of 100 cases, found positive for MP on any of the methods, 75 % were males, 25% were females with mean age of 38 + 5, ranged from 22 to 50 years in both gender. Positive MP was plasmodium falciparum in 100 (100%) cases. Pre-treatment MP was found positive on ICT in 88 (88%) cases while on MP film 95 (95%) and mean MP index was 0.49% ranging from 0 to 3.5%. Post-treatment MP was found positive on

ICT in 88 (88%) cases while on MP film 14 (14%) and mean MP index was 0.04% ranging from 0 to 1.8% (table-I & II).

Pre-treatment diagnostic accuracy of ICT revealed sensitivity of 87% and specificity of 97%, as compared to pre-treatment microscopic film method (table-III & figure).

Table-I: Baseline qualitative characteristics of malarial patients, found positive for MP on any of the methods (n=100).

Parameters	n (%)
Male	75 (75)
Female	25 (25)
Pre-treatment MP positive on ICT	88 (88)
Pre-treatment MP positive on MP film by microscopy	95 (95)
Type of MP (Falciparum)	100 (100)
Post-treatment MP positive on ICT	88 (88)
Post-treatment MP positive on MP film by microscopy	14 (14)

Table-II: Quantitative characteristics of malarial patients, found positive for MP on any of the methods (n=100).

Parameters	Mean (Ranges)
Age (Years)	38 (22-50)
Pre-treatment MP index %	0.49 (0-3.5)
Post-treatment MP index %	0.04 (0-1.8)

Table-III: Pre-treatment diagnostic accuracy of ICT in comparison to microscopic film method (n=250).

Test ICT	Malaria Present (Microscopy film method)		Total
	Present	Absent	
Positive	83=a	5=c	88= a+c
Negative	12=b	167=d	179=b+d
Total	95= a+b	172= c +d	267
Sensitivity of ICT	100 x a / (a+b) = 87%	a = true positives b = false negatives c = false positives d = true negatives	
Specificity of ICT	100 x d / (c+d) = 97%		

DISCUSSION

Due to high mortality rate of malaria, especially the falciparum species and its high prevalence in African countries with limitations of the microscopic and molecular methods, the use of rapid diagnostic methods seems necessary for timely diagnosis of this deadly infection. Therefore, many efforts have been made to detect malaria by easy and rapid but reliable methods. Recently, many rapid diagnostic tests have been developed for rapid detection of malarial parasite. These assays involve the use of fluorescence microscopy, ICT and molecular methods like PCR (polymerase chain reaction)[7-9]. Although the gold standard for diagnosis of malaria remains microscopy yet ICT provides an opportunity to diagnose malaria earlier in the course of disease, and facilitate an appropriate therapy, thereby reducing mortality[10].

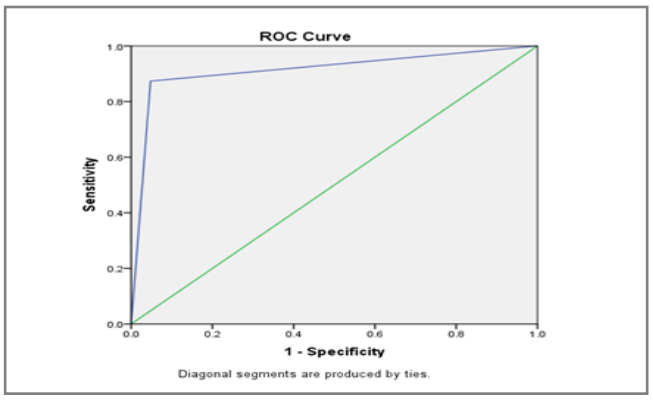

Figure: Pre-treatment ICT sensitivity was 87 % and specificity was 97% (n=250).

Current study was performed to compare ICT with microscopic method of malarial parasite detection specially plasmodium falciparum and to determine diagnostic accuracy of ICT method considering microscopy as gold standard. The results showed a sensitivity of 87% and specificity was 97% for ICT compared to microscopic method. These findings are consistent with a number of other studies. Study by Dash in India revealed ICT sensitivity 87.5% and specificity 87.3% for plasmodium falciparum[3]. Muhammad *et al*. showed that ICT yielded a very high sensitivity (96.1%) and specificity (95.7%) for malaria. The false positive rates and false negative rates were also very low, being 4.3% and 3.9% respectively[10]. Zeb and coworkers studied two rapid test techniques i.e. ICT and Opti MAL devices and recorded 100% sensitivity and specificity for plasmodium falciparum while 75 to 87.5% sensitivity and 100% specificity for vivax infection[11]. Sheikh *et al*, showed that RDTs have same

sensitivity and specificity compared with routine microscopy[12]. A detailed study by Batwala *et al* in 2010 in rural health care centres at Uganda found that the sensitivity of presumptive diagnosis of malaria based on axillary temperature, microscopy and rapid diagnostic test were: 42.6%, 85.1% and 97.9% respectively, the corresponding specificity rates were found to be 73.1%, 93.7% and 74.7% respectively, thus the malarial antigen based tests demonstrated a superior sensitivity compared to microscopy but lower specificity[13]. Similarly, a recent large study in three African countries recorded a high sensitivity of 94.3% for RDT but lower specificity of 41.6%[14]. Findings of high sensitivity and low specificity are not uncommon in areas of high malaria transmission[15].

Anti HRP-II based RDTs are the preferred options for tropical areas, where Plasmodium falciparum is responsible for >95% of malaria infections. The most common reason for low specificity is the fact that HRP-II clears slowly, with the result that infections that have been cured more than 2-3 weeks earlier may still test positive[16-18]. This is also supported by our study results, causing a detection of post treatment MP on ICT in 88% cases as compared to 14% on microscopy. Post-treatment positive results on ICT may sometimes be mislead regarding response of treatment, but it may be helpful in identifying duration of clearance of antigen from the body. Also in cases where treatment is started earlier on the suspicion of malaria by clinical features, ICT can still detect MP after 2 to 3 weeks of the start of malaria and help in identifying the type of malaria by detecting anti HRP-II specific for falciparum alone and anti aldolase antibodies/ pan line for all other species of malaria.

LIMITATION OF STUDY

In our study all the cases were of falciparum malaria. Validation of the diagnostic accuracy of ICT can also be compared with microscopic film method in other areas where different species of malarial parasite do exist and in cases of mixed malarial infections.

CONCLUSION

Considering consistent findings of all these studies and results of our study in favour of ICT test especially for falciparum malaria, It is concluded that immunochromatographic technique provides rapid, useful, sensitive, user friendly and practical alternative to slide microscopy for the diagnosis and guiding management of malaria specially in remote areas, despite the highlighted shortcomings of false-negative and false-positive results.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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