THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Performance of First Response HRP2 Based Malaria Rapid Diagnostic Test in Whole Blood and Saliva in Lagos State

Elendu Judith Anurika
Department of Medical Microbiology and Parasitology
College of Medicine, idi- araba, University of Lagos, Nigeria
Department of Zoology, University of Lagos, Akoka, Lagos, Nigeria
Oyibo W. A.
Department of Medical Microbiology and Parasitology
College of Medicine, Idi-Araba, University of Lagos, Nigeria

Abstract:
The study assessed the performance of First Response Histidine Rich Protein 2 (HRP2) based malaria rapid diagnostic test (MRDT) in whole blood and saliva samples, in order to assess the usefulness of saliva as an alternative sample to blood in malaria diagnosis. A total of 1026 blood samples and 186 saliva samples (total RDT positive sample) were collected from patients who attended the clinic, and fell within the inclusion criteria at St Mathew's Hospital, Amukoko, Lagos State. Microscopy and rapid diagnostic test were carried out on the whole blood samples and saliva.
The MRDT tested demonstrated a high sensitivity of 95% and a high specificity of 98.7% at a 95% confidence interval(p=0.445) for whole blood while saliva samples demonstrated a low sensitivity of 9.4% and high specificity of 100%. There was no significant difference between microscopy and First Response HRP 2 (χ^2, P=0.05) and as such, it is a good diagnostic tools for malaria while saliva serves as a promising possible means of non-invasive technique in malaria diagnosis.

Keywords*: rapid diagnostic test, saliva, sensitivity, specificity, microscopy and malaria*

1. Introduction

First response malaria rapid diagnostic test are commercially available tests that allows the rapid diagnosis of malaria, thereby enhancing prompt diagnosis and treatment which are vital keys in the reduction of malaria morbidity and mortality (18). MRDTs are currently being augmented with microscopic examination of blood smears (microscopy) which still remains the gold standard for *Plasmodium* specie detection. However, microscopy does need, well qualified laboratory scientist, maintenance of the microscope and regular supply with consumables; conditions, which are difficult to sustain in developing and under developed countries (12).
Malaria rapid diagnostic test (MRDT), detect parasite within a threshold of 100 parasites/µl of blood, targeting malaria antigens such as histidine rich protein 2(HRP2), Parasite Lactate Dehydrogenase (PLDH) and Aldolase. They are easy to perform, do not require extensive training or equipment, and are currently being used to detect antigens of *Plasmodium* species in blood, serum, plasma, etc, to supplement microscopic evaluation of blood smears, in the management of tropical febrile disease (11, 15). *Plasmodium falciparum* HRP2 antigen has been detected in erythrocytes, serum, plasma, cerebrospinal fluid and even urine and saliva (4, 13).
However, there is continued negative impact to malaria diagnosis due to; inaccurate microscopic evaluation of blood smears, which have resulted in misdiagnosis and misclassification of malaria severity (8), blood taboos and increased risk of accidental infections (albeit minor) due to needle pricks. In non specialized laboratories, microscopic evaluation of blood smears is slow and may lead to late diagnosis and treatment, which contributes to high mortality rates (7).
Thus, the objectives of this research are to evaluate the performance of HRP2 RDT as a diagnostic tool, in saliva and whole blood, and determine the usefulness of saliva in malaria diagnosis.

2. Materials and Method

2.1. Study Area and participant

The study was conducted during November 2010 – May 2011 at St Mathew's primary health centre/ hospital, Amukoko, in Apapa / Iganmu Local Council Development Area in Ajeromi Ifelodun Local Government Area, in Lagos State, Nigeria, West Africa.
Case report forms were used to recruit participants at the hospital, after obtaining informed written consent from individuals, parents/ guardians of children below 18 who presented with clinical symptoms of malaria such as fever on site or within the past three days, body pain/ joint pain, head ache, vomiting, loss of appetite, weakness, etc. ranging from children below 5 years of age to adults. The exclusion criteria were those with other complaints unrelated to malaria and complicated malaria.

2.2. Sample Collection

Venous blood (approximately 3ml) was collected from each patient and placed into a properly labelled (with patient's identity number) EDTA container, for malaria diagnosis by 2 methods: microscopy and RDT; immediate slide containing both a thick smear and a thin smear was provided (according to the world health organization standard) to the clinical staff for immediate staining and observation, while the remaining blood samples was transported to the microscopy laboratory at the Department of Medical Microbiology and Parasitology, College of Medicine, Idi-Araba for further testing.

Saliva samples (approximately 1 ml) were collected by spitting, in properly labelled sterile bottles, from patients who were positive for malaria by rapid diagnostic test, done immediately, at the site of collection; A total of 1026 whole blood samples and 186 saliva samples (total number of positive sample by rapid diagnostic test) were collected.

2.3. Sample Processing

Malaria diagnosis by two commercial RDTs SD Bioline Malaria Ag (Standard Diagnostic Incorporation, Hagal-Dong, Korea) ® catalogue no 082043 and 082065 with expiry date march, 2012 and September, 2012 respectively; First Response Malaria (HRP2) Antigen (Premier Medical, India) ® catalogue no 56H2509 and expiry date may, 2012, were performed on all blood samples using the methods/ procedure described by the manufacturer.

In saliva samples, no further processing was done; the saliva was properly mixed and the same MRDT kits were used (SD Bioline Malaria Ag and First Response Malaria (HRP2) Antigen) following the manufacturer's instruction. All RDT kits were stored as directed by the manufacturer and underwent quality assurance testing at the World Health Organization quality assurance testing center at the Department of Medical Microbiology and Parasitology, College of Medicine, Idi-Araba; after which, two slides were prepared designated read and archive for the study purpose, excluding the one done at the study site for the clinical staff; each slide having both a thick and thin blood smear and stained with 3% giemsa stock solution, for 45 minutes and examined by two independent qualified microscopist, according to world health organization study protocol (19).

2.4. Result Interpretation

The presence of control line and test lines indicated a positive result for *P. falciparum* while, the presence of only the control line indicated a negative result. The absence of a control line was interpreted as invalid and the test was repeated. Positivity of the samples were graded as 1+, 2+, and 3+, depending on the intensity of the test line when compared to the control line.

2.5. Validation And Control

While performing the RDTs, I and reader 2 were blinded to each of the other measurements collected during the study. Care was taken to ensure that technicians using the rapid diagnostic device were blinded to patient histories and examinations, WBC determination and patient demographics. In all cases the results of the RDT (First Response HRP2) were determined prior to diagnostic microscopy with strict blinding between the rapid test results and technicians performing the microscopy

2.6. Data Analysis

Data were entered and verified using the Microsoft excel format and analysed using SPSS version. Over all agreement of reliability of RDT readings was calculated. Using microscopy as the reference, Proportions were assessed for statistical significance using the Pearson chi-square test. A p- value <0.05 was considered significant.

3. Results

Of the 1026 patients sampled during the study, 402 (39.2%) of them were males while, 624 (61%) were females; with a mean age of 25.6 (SD=16.7, range 0.1- 80 years).

181 of the study sample (17.6%) {95% CI 15.4-20.1%) were positive for malaria by microscopy with *Plasmodium falciparum* being the most predominant specie (97.8%) and 2.2% represented other species of which *P.malariae* consist of 25%. The trophozoite stage was the most frequent (93.1%) while gametocytes represented 6.0%.

183 (17.8%) were positive for malaria by First Response HRP 2 and 17 out of 186 (9.1%) saliva samples were positive for malaria by First Response RDT.

The performance of the individual test (First Response) varied in blood and saliva (table 1).

Blood				**Saliva**			
First Response	**Microscopy**		**Total**	**First Response**	**Microscopy**		**Total**
	Negative No (%)	**Positive No (%)**	**No (%)**		**Negative No (%)**	**Positive No (%)**	**n=(1026) No (%)**
Negative	834(81.3)	9 (0.9)	843(82.2)	**Negative**	845(82.4)	164(16)	1009(98.3)
Positive	11(1.1)	172(16.8)	183(17.8)	**Positive**	0(0)	17(1.7)	17(1.7)
Total	845(82.4)	181(17.6)	1026(100)	**Total**	845(82.4)	181(17.6)	1026(100)

Table 1: Performance of First Response RDT Using Blood and Saliva

The comparative performance characteristics of First Response HRP2 in blood and saliva varied with sensitivity 95% and 9.4% , specificity 98.7% and 99.5%, positive predictive value 94% and 99.5%, negative predictive value 98.7% and 83.7% in whole blood and saliva respectively, at a 95% confidence interval(p=0.445).

226 (22.0%) of the 1026 samples were diagnosed as febrile (>37.5°C), with a mean temperature of 36.9°C (SD 0.96, range 33.6°C - 41.0°C), of which 59(26.1%) were positive by microscopy, 57(25.2%) were positive by First Response and 9(4.0%) were positive for saliva.

Age group 10-19, showed the highest prevalence {45(24.9%), 45(24.2%)} in blood by microscopy and First Response RDT respectively but the difference was not significant; whereas, the highest prevalence {6(35.3%), in saliva samples was seen in age group 20-29.

Parasitemia levels ranged from 3 to 217,135, with a mean of 9,316.6. RDT proved highly sensitive at low levels of parasitemia as sensitivity in detecting *P. falciparum* was 95.1% when parasite density was between 1-200 parasites/µl of blood in First Response RDTs.

A sensitivity of 9.8% was also observed in saliva samples at low parasite density; which increased as parasite density increased, although this difference was not statistically significant (p=0.3852).

4. Discussion

Over time, alternative tests for malaria diagnosis have been developed in order to support the performance of microscopy and to facilitate prompt and accurate diagnosis and timely intervention.

This study demonstrated a low prevalence (17.7%), First Response HRP2 demonstrated a high sensitivity (95 %) and specificity (98.7%) in blood, which is in agreement with previous studies (9, 2) and the world health organization standard for all RDTs (18).

The false negative observed by the RDT was attributed to very low parasite density and presence of other *Plasmodium species*, while the false positive was attributed to cross reaction with auto antibodies /rheumatoid factors, or persistence of circulating HRP2 antigen either due to sequestration or incomplete treatment. Blood specificity was high, showing that the test is reliable in detecting the absence of *Plasmodium falciparum* antigen which agreed with past studies (6, 16). Issues concerning over diagnosis and waste of therapeutic drugs (ACT) did not arise.

First Response HRP2 RDT also demonstrated high frequency (95.8%) at detecting *Plasmodium falciparum* antigen at low parasitemia.

Evaluation of saliva as a possible means of diagnosis with HRP2 based RDTs has revealed a high specificity (99.5%), a very low sensitivity (9.4%), a high negative predictive value (83.7%) and a positive predictive value (99.5%) for First Response HRP2 RDT, re affirming the results gotten from other research (2, 13) with slight deviation. Saliva is able to detect the absence of *Plasmodium falciparum* effectively, thus reducing the possibility of false positive result.

Low sensitivity of First Response HRP2 RDT in saliva was due to limitations of the commercially available kit used, which is designed to detect higher levels of *Pf*HRP2 in whole blood or plasma, than is found in saliva. However, the result showed that the antigen can be detected in saliva, which is a non invasive method.

Nevertheless, improvement of detection method is of primary importance before saliva can be reliably applied for alternative diagnosis of malaria parasites or evaluation of malaria control measures such as vaccine efficacy.

5. Acknowledgement

We would like to thank the staff of St Mathew's Catholic Hospital Amukoko, Lagos State. All the standard diagnostic RDTs used were supplied by Codix Pharm Limited.

6. Refernces

1. Bell, D, Peeling, R. W, (2003). Evaluation of Rapid Diagnostic Tests: Malaria. Nature Review Microbiology 4(9): 34-38.
2. Buppan, P, Putaporntip, C, Pattanawong, U, Seethamchai, S, Jongwutuwes, S, (2010). Comparative Detection of Plasmodium vivax and Plasmodium falciparum DNA in Saliva and Urine Samples from Symptomatic Malaria Patients in a Low Endemic Area. Malaria Journal 9:72-76.
3. Estevez, P. T, Judith, S, Nwakanma, D. C, Sheila, W, David, J. C , Chris, J. D, (2011). Human Saliva as a Source of Antimalaria Antibodies to Examine Population Exposure to Plasmodium falciparum. Malaria Journal 10:104-112.
4. Genton, B, Paget, S, Beck, H, P, Gibson, N, Alpers, M. P, Hii, J, (1998). Diagnosis of Plasmodium falciparum Infection Using Para Sight ®- F Test in Blood and Urine of Papua , New Guinean Children. Southeast Asian Journal of Tropical Medical Public Health 29:35-40.
5. Grenade, T. C, Phillips, S. K, Parek, B, Gomez, P, Kitson-Piggott, W, Oleander, H, Mahabir, B, Les, W, Lee-Thomas, S, (1998). Detection of Antibodies of Human Immunodeficiency Virus Type 1 in Oral Fluids: A Large Scale Evaluation of Immunoassay Performance. Clinical Diagnostic Laboratory and Immunology 5:171-175.
6. Jobiba, C, Jacek, S, Ben, C, Carl, C, Victoria, E, Miguel, S .J, John, S, Doreen, A, Don, M, (2010). Comparative Field Performance and Adherence to Test Results of Four Malaria Rapid Diagnostic Tests Among Febrile Patients More Than Five Years of Age in Blantyre Malawi. Malaria Journal 9:209.
7. Kain, K. C, Harrington, M. A, Tennyson, S, Keystone, J. S, (1998). Imported Malaria: Prospective Analysis of Problems in Diagnosis and Management. Clinical Infectious Diseases 27:142-149.
8. Makler, M, Palmer, C. J, Ager, A.l, (1998). A Review of Practical Techniques for the Diagnosis of Malaria. Annals of Tropical Medical Parasitology 92:419-433.

9. Maltha, J, Phillipe, G, Emmanuel, B, Lieselote, C, Marjan, V. E, Jan, J, (2010). Evaluation of Rapid Diagnostic Test (Care Start Tm Malaria HRP-2 / PLDH (Pf/Pan) Combo Test) for the Diagnosis of Malaria in a Reference Setting. Malaria Journal 9:171.
10. Maltha, J, Philippe, G, Lieselotte, C, Emmanuel, B, Marjan, V. E, Cathrien, B, Jan, J, (2011). Evaluation of the Rapid Diagnostic Test SD FK 40 (Pf-PLDH/Pan-PLDH) for the Diagnosis of Malaria in a Non Endemic Setting. Malaria Journal 10:7-18.
11. Moody, A, Hunt-Cooke, A, Gabbett, E, Chiodini, P, (2000). Performance of the Optimal Malaria Antigen Capture Dipstick for Malaria Diagnosis and Treatment Monitoring at the Hospital for Tropical Diseases. London British Journal of Hematology 109(4):891-894.
12. Murray, C. K, Gasser, R .A .Jr, Magill, A. J, Miller, R. S, (2008). Update on Rapid Diagnostic Testing for Malaria. Clinical Microbiology Reviews 21(1): 97-110.
13. Nana, W. O, Andrew, A. A, Winston, A, Stella, B, Jonathan, K. S, (2008). Short Report: Detection of Plasmodium falciparum Histidine Rich Protein II in Saliva of Malaria Patients. American Journal of Tropical Medical Hygiene 78(5): 733-735.
14. Nokes, D .J, Enquselassie, F, Nigeria, W, Vyse, A. J, Cohen, B .J, Brown, D. W, Cuts, F. T, (2001). Has Oral Fluid the Potential to Replace Serum for the Evaluation of Population Immunity Levels? A Study of Measles, Rubella and Hepatitis B in Rural Ethiopia. Bulletin World Health Organization 30:1076-1079.
15. Singh, N, Saxena, A, Sharma, V.P, (2002). Usefulness of an Inexpensive Paracheck Test in Detecting Asymptomatic Infectious Reservoir of Plasmodium falciparum During Dry Season in an Inaccessible Terrain in Central India. Journal of Infection 45(3):165-168.
16. Wanna, C, Thane, W, Ronnatrai, R, Kesara, N. B, (2011). Evaluation of Rapid Diagnostics for Plasmodium falciparum and P.vivax in Make Sot Malaria Endemic Thailand. Korean Journal of Parasitology 4 (91) :33-38.
17. Wilson, N. O, Adjei, A. A, Anderson, W, Baidoo, S, Stiles, J.K, (2008). Detection of Plasmodium falciparum Histidine – Rich Protein II in Saliva of Malaria Patients. .American Journal of Tropical Medical Hygiene .78:733-735.
18. World Health Organization (2010a). Malaria Case Management: Operation Manual. Geneva, Switzerland.
19. World Health Organization (2010b).Malaria Diagnosis: WHO Guidelines and Their Implementations. Geneva, Switzerland