



Diagnostic Performance of Hrp2 Based Diagnostic Test Kits and Frequency of Pfhrp2 Gene Deletion in *Plasmodium falciparum* Isolates of Osogbo, Southwestern Nigeria

A. S. Nassar¹, A. S. Bakarey^{2*}, A. A. Abdulazez³ and O. O. Fayemi¹

¹Department of Medical Laboratory Sciences, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

²Institute for Advanced Medical Research and Training College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

³Department of Medical Laboratory Science, University of Medical Science, Ondo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors designed the study, performed the statistical analysis, wrote the protocol and authors ASN and ASB wrote the first draft of the manuscript. Authors ASN and ASB managed the analyses of the study. Author AAA and OOF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2021/v33i730870

Editor(s):

(1) Prof. Ibrahim El-Sayed M. El-Hakim, Riyadh College of Dentistry and Pharmacy, Saudi Arabia.

Reviewers:

(1) Amanze Ikwu, University Hospitals Plymouth NHS Trust, United Kingdom.

(2) Vijaya Shivanna, Adichunchanagiri University (ACU), India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/62106>

Original Research Article

Received 01 September 2020

Accepted 02 November 2020

Published 27 March 2021

ABSTRACT

Introduction: The introduction of *P. falciparum* encoded HRP-2 based malaria Rapid Diagnostic Test (RDT) kits is widely accepted in Nigeria and worldwide as a simplified form of diagnosis and a cheaper alternative to the microscopy technique (gold standard). However, deletion of Pfhrp2 gene contributes to false negative results and large number of such deletions has been reported in advanced countries thereby highlighting the importance of surveillance to detect such deletions in our local environment.

Methodology: Microscopy as well as RDT techniques (using Rapid malaria test kit: SD BIOLINE Malaria Ag P.f/Pv, South Korea) were carried out on the blood samples of three hundred and

*Corresponding author: E-mail: asbakarey@com.ui.edu.ng, drbakarey@yahoo.com;

twenty-three (323) febrile subjects attending Ladoke Akintola University Teaching Hospital, Osogbo, Osun State Nigeria. PCR analysis was also conducted on 50 blood samples that were positive for microscopy but negative for RDT.

Results: The results from the study revealed that microscopy had a sensitivity of 99% and specificity of 99.2%. The RDT however had a sensitivity of 100% and a specificity of 60.1%. Fifty (50) samples that were positive for microscopy but negative for RDT were subjected to further PCR examination to detect the possible deletion of the *Pfhrp-2* gene and the result revealed that the gene was present in 39 (78%) of the blood samples while remaining 11 (22%) samples lacked the gene which could possibly be the reason for the negative results obtained using the RDT kits.

Conclusion: This study provides evidence of low level of presence of *Pfhrp-2* gene deletion of *Plasmodium falciparum* parasites in our healthcare facility setting in Osogbo, Nigeria.

Keywords: RDT and PCR; *Plasmodium falciparum* histidine rich protein; microscopy; Nigeria.

1. INTRODUCTION

Rapid Diagnostic Test Kits (RDTs) are individual laboratory devices that can detect *Plasmodium*-specific antigens in a small volume (5 to 10µl) of fresh blood using lateral flow immunochromatography [1]. RDTs offer a feasible Malaria alternative to microscopy as they do not require a laboratory or special equipment, simple to use, and provide a positive or negative result within 20 minutes [1,2]. Malaria Rapid Diagnostic Tests [mRDTs] have made it possible to obtain diagnostic results quickly and provide treatment in a timely manner [2]. The availability of RDTs and the scale of their use globally have increased from 46 million in 2008 to 319 million in 2013 [1-3].

It has been reported that HRP2-based RDT gave false-negative results when compared to microscopy and PCR respectively [3]. A lot of concerns have been raised that such false-negative results may reflect presence of parasites with *Pfhrp2* gene deletions or mutations [3]. There has long been evidence of *P. falciparum* parasites lacking the *Pfhrp2* gene in regions of South America and public health authorities have always cautioned against using HRP2-based RDTs in the Amazon River basin [4]. In the past few years, reports have emerged from *Pfhrp2* mutations or deletions in Africa and India, leading to false negative RDT results [5]. The existence of parasites lacking *Pfhrp2* would affect RTD accuracy in a broader range of malaria-endemic regions and would have significant implications for RDT implementation, clinical case management, and malaria control efforts [6]. Presently, there are limited documented reports in Africa to prove the true extent of such gene deletion.

Most of the commercially available malaria RDT kits target the *P. falciparum* specific histidine rich

protein-2 (*PfHRP2*) because it is a highly stable and specific biomarker. Recent studies have shown that the genes that encodes for this protein and its paralog, histidine-rich protein-3 (*Pfhrp3*), are absent in parasites from the Peruvian Amazon Basin [6]. Other related studies from countries such as Mali, Senegal and India have also suggested that *P. falciparum* isolates circulating in those regions may also lack *Pfhrp2* genes [6,7]. Therefore, this study aimed at assessing diagnostic performance of hrp2 based diagnostic test kits and frequency of pfhrp2 gene deletion in *Plasmodium falciparum* isolates of Osogbo, Southwest, Nigeria.

2. METHODOLOGY

2.1 Study Area and Geographical Location

The study was conducted in Osogbo, Osun State, South-west Nigeria. Osogbo, the capital of Osun State lies between longitude 4° 34'E and latitude 7° 46'N. The town's land mass is approximately 47 km² with a population of 156,694. It is occupied by an admixture of few other ethnic groups, but the predominant ethnic group is the Yorubas. The town also plays host to foreigners on daily basis due to the presence of Osun-Osogbo groove, an internationally recognized tourist centre. The town has primary, secondary, tertiary and comprehensive healthcare centres [8].

2.2 Blood Sample Collection and Processing

Blood samples were collected from consenting malaria suspected febrile patients ranging between age (1-65years) attending Ladoke Akintola UNIVERSITY of Technology Teaching Hospital (LAUTECH) Osogbo, Nigeria. Pregnant

women and those on anti-malaria therapy were excluded from the study. Rapid diagnostic tests (RDTs) as well as microscopic examinations were conducted at the Microbiology and Parasitology laboratory of LAUTECH Teaching Hospital while Polymerase Chain Reaction (PCR) analysis was carried out at GeneLab, Ibadan.

RDT test was conducted immediately after collection of blood samples and processed according to the manufacturer's (SD BIOLINE Malaria Ag P.f/Pv, South Korea) instruction. About 2-3 ml of blood samples was collected into Ethylene diamine tetra acetate (EDTA) anticoagulants tubes after which thick and thin blood films were made from the blood specimen and then stained using Giemsa staining technique [9]. Blood samples that were microscopy positive but RDT negative were selected and subjected to PCR analysis to detect the PfHRP-2 gene. PCR amplification was carried out on the extracted DNA to detect the possible deletion of *Pfhrp-2* exon-1 and exon-2 genes according to Jena bioscience DNA technique. Also, both positive and negative controls were prepared along with the samples.

Data collected from the study were analyzed using Statistical Package for Social Science (SPSS) version 17.0 (SPSS Inc., Chicago, USA). P-value less than 0.05 ($p < 0.05$) was considered statistically significant. The findings of the study revealed the prevalence of HRP2 based RDT negative *P. falciparum* among natural isolates of Osogbo, Osun State, Nigeria during the rainy season when malaria was highly endemic.

3. RESULTS

Comparison of Microscopy and Rapid Diagnostic Test RDT with respect to their results as shown in Table 1, Microscopy used for the diagnosis of malaria infection indicated that the sensitivity was 99% when compared with RDT which had 100%. Also the specificity using Microscopy was 99.2% as against 60.1% recorded for RDT. Furthermore, the positive predictive value (PPV) was 99.5% for Microscopy while a lower value of 58.1% was found for RDT. Finally, negative predictive value was 98.5% for Microscopy while that for RDT was higher in the range of 100% (Table 1).

Of the 323 samples tested for Microscopy as a gold standard, 199 were positive for Malaria. All the 199 malaria positive samples were retested

with RDT of which 115 were positive. However, 50 samples that were positive for microscopy but negative for RDT were further subjected to PCR evaluation to detect the possible deletion of the *Pfhrp-2* gene PCR of which 39(78%) were positive for PFHRP-2 as indicated in the flow chart (Fig. 1).

4. DISCUSSION

The World Health Organization (WHO) recommends prompt diagnosis and treatment of malaria especially in endemic regions as a vital way of combating the disease. In order to achieve these goals however, there has been increased dependence on the use of Rapid Diagnostic Test kits especially in rural communities where laboratory facilities are absent [10]. Majority of the commercially available RDT kits target the *P. falciparum* specific HRP-2 as it is a stable and specific biomarker that is available at all stages of the parasites life cycle. Malaria in Nigeria is mostly due to *P. falciparum* infections and in majority of the rural areas where the disease is highly endemic, the use of RDT is the primary choice for diagnosing malaria in case management [2]. However, substantial evidences of PfHRP2-based RDT failure in diagnosing *P. falciparum* infections in recent years from various parts of South America, Asia and Africa including Nigeria [11] are quite worrisome.

Considering the frequency of *Plasmodium falciparum* infection in the study area using microscopy and RDT techniques, the present study reported that 199 (61.6%) were positive for *Plasmodium falciparum* using microscopy while 84 (26%) of this number were negative using RDT (Fig. 1). The result showed mono-infection of *P. falciparum* in the study area and no other species of malaria parasite was detected by microscopy. The diagnostic performance of microscopy and RDT techniques taking sensitivity and specificity into consideration showed 99% sensitivity and 99.2% specificity with microscopy (as gold standard) and RDT technique recorded 100% sensitivity and 60.1% specificity respectively (Table 1) although, previous study conducted in the same study area showed RDT sensitivity of 62% and specificity of 87.4% [12].

The study conducted on the frequency of *Plasmodium falciparum* HRP2 gene deletions among febrile subjects in Osogbo and its environment was found to be the first of its kind

since no document have recorded similar study for the prevalence of *Pfhrp2* and *Pfhrp3* genes in natural *Plasmodium falciparum* population which is known to be endemic in the study area. This study was designed to identify false RDT negative blood samples by microscopy and PCR. Out of the 84 blood samples that were positive for microscopy and negative for RDT, 50 of these samples were randomly selected based on heavily parasitized samples and then subjected

to PCR amplification to determine the frequency of the *Pfhrp2* gene deletion (Fig. 1). The present study shows that 39 (78%) of the samples subjected to PCR technique were positive for *Pfhrp2* gene in the study area while 11 (22%) were negative for *Pfhrp2* gene which probably indicated signs of gene deletion of *Pfhrp2* in *Plasmodium falciparum* in this environment (Fig. 1).

Table 1. Sensitivity, specificity, positive and negative predictive value of RDT and microscopy results

Variable	Sensitivity	Specificity	PPV	NPV
Microscopy	99.0%	99.2%	99.5%	98.5%
RDT	100%	60.1%	58.1%	100%

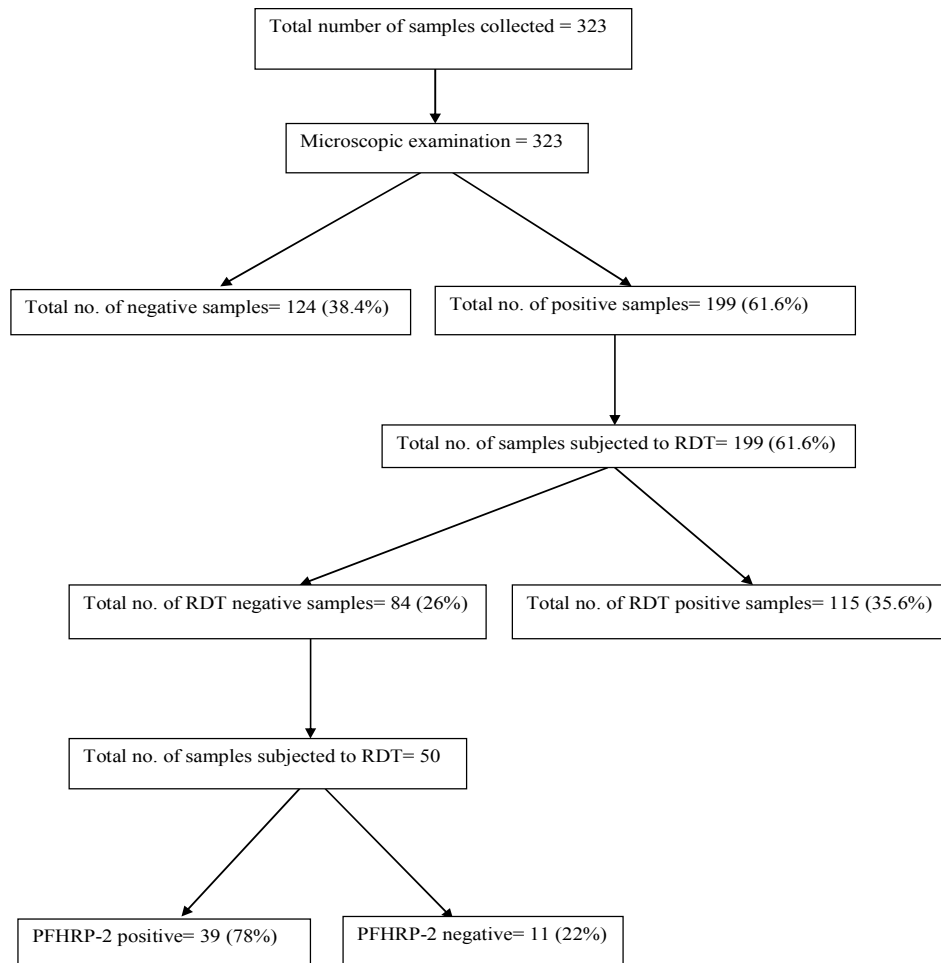


Fig. 1. Flow chart showing the summary of all the steps involved during the course of the study

The result from the study in comparison with an earlier study carried out in India by Praveen *et al.* [13] reported that 0-25% and 0-11% were recorded from two different study areas in India. Our result is also in consonance with Pati *et al.* [11] thought that the prevalence of high proportion of *Pfhrp2* was recorded during dry and rainy season unlike our study which was carried out only during rainy season with corresponding lower results. The strength of this study is that the samples were collected during the transmission period of malaria. However, the study has some limitations: Only symptomatic patients were selected and screened for malaria infection during this period, though previous report indicated that the occurrence of *Pfhrp2* gene deletion has also been reported among asymptomatic patients [13]. Moreover, the investigation was not carried out on HRP3 population and PCR amplification was not carried out among RDT positive samples.

5. CONCLUSION

The findings of the study revealed the prevalence of HRP2 based RDT negative *P. falciparum* among natural isolates of Osogbo, Osun State, Nigeria during the rainy season when malaria is highly endemic. The failure of RDT in detecting *P. falciparum* infection may probably be due to undetectable level of HRP2 antigen. Our study therefore suggests that there should be periodic evaluation of RDT performance and molecular surveillance in order to ensure reliable performance of RDTs and also to monitor any changes in the level of *Pfhrp2* gene deletion in *Plasmodium falciparum* in different parts of Nigeria. It is however advocated that periodic surveillance should be conducted in order to monitor the reliability of RDT test kits. Furthermore, all RDT negative test results should be subjected to microscopic examination before drawing conclusion on such malaria test result(s).

CONSENT

As per international standard or universal best practices, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The ethical approval for the study was obtained from the ethical committee of Ladoke Akintola University of Technology(LAUTECH) Teaching Hospital, Osogbo, Nigeria while parental assents

were given for the under aged participants. The ethical approval was obtained from LAUTECH Teaching Hospital Institutional Review Board (LTH IRB) committee with the number (LTH/EC/2015/11/236). This study commenced after obtaining ethical clearance from the appropriate stake holders.

ACKNOWLEDGEMENTS

We are grateful to all the participants who consented to take part in this study by giving us their full cooperation. Our gratitude also goes to the management and staff of Ladoke Akintola University of Technology Teaching hospital, Osogbo and GeneLab, Ibadan for the use of their laboratory facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wongchotigul V, Suwanna N, Krudsood S, Chindanond D, Kano S, Hanaoka N, et al. The use of flow cytometry as a diagnostic test for malaria parasites. Southeast Asian Journal of Tropical Medicine and Public Health. 2004;35:552-559.
2. World Health Organization. Universal access to malaria diagnostic testing-an operational manual Geneva; 2014. Available:<http://www.who.int/malaria/publications/atoz/9789241502092/en/>.
3. Christina T Kozycki, Noella Umulisa, Stephen Rulisa, Emil I Mwikarago, Jean Pierre Musabyimana, Jean Pierre Habimana et al. False-negative malaria rapid diagnostic tests in Rwanda: Impact of *Plasmodium falciparum* isolates lacking *hrp2* and declining malaria transmission. Malaria Journal. 2017;16:123.
4. Abba K, Deeks JJ, Olliaro P, Naing CM, Jackson SM, Takwoingi Y et al. Diagnostic tests for diagnosing uncomplicated *P. falciparum* malaria in endemic countries. 2011;(7):CD008122. DOI: 10.1002/14651858.CD008122.pub2
5. Khalid B Beshir, Nuno Sepúlveda, Jameel Bharmal, Ailie Robinson, Julian Mwanguzi, Annette Obukosia Busula et al. *Plasmodium falciparum* parasites with histidine-rich protein 2 (*pfhrp2*) and *pfhrp3* gene deletions in two endemic regions of Kenya. 2017;7:14718.

6. Rachid VGM, Akniyi OS, Silva-Flannery L, Lima BDR, Macedo de Oliveira A, Goldman IF. Histidine-rich protein 2 (*Pfhrp-2* and *Pfhrp-3*) gene deletions in *P.falciparum* isolates from selected sites in Brazil and Bolivia. PLoS. 2017;12(3): e0171150.
7. Giselle Maria Rachid Viana, Sheila Akinyi Okoth, Luciana Silva-Flannery, Danielle Regina Lima Barbosa, Alexandre Macedo de Oliveira, Ira F Goldman et al. Histidine-rich protein 2 (*pfhrp2*) and *pfhrp3* gene deletions in *Plasmodium falciparum* isolates from select sites in Brazil and Bolivia PLoS One. 2017;12(3):e0171150.
8. Monsuru Adebayo Adeleke, Wasiu Olalekan Adebimpe, Abdul Wasiu Oladele Hassan, Sunday Olukayode Oladejo, Ismail Oiaoye, Ganiyu Olatunji Olatunde. Taiwo Adewole Larval habitats of mosquito fauna in Osogbo metropolis, Southwestern Nigeria. Asian Pac J Trop Biomed. 2013; 3(9):673–677.
9. Monica Cheesbrough 2010 Tropical Health Technology, Norfolk Publisher: Cambridge University; December 2010. DOI:<https://doi.org/10.1017/CBO9780511581304>
10. Orish VN, De-Gaulle VF, Sanyaolu AO. Interpreting rapid diagnostic test (RDT) for *Plasmodium falciparum*. BMC Res Notes. 2018;11:850 (2018). Available:<https://doi.org/10.1186/s13104-018-3967-4>
11. Pati P, Dhangadamajhi G, Bal M. High proportions of *pfhrp2* gene deletion and performance of HRP2-based rapid diagnostic test in *Plasmodium falciparum* field isolates of Odisha. Malar J. 2018;17: 394. Available:<https://doi.org/10.1186/s12936-018-2502-3>.
12. Ojurongbe O, Adegbosin OO, Taiwo SS. Assessment of clinical diagnosis, microscopy, rapid diagnostic tests and polymerase chain reaction in the diagnosis of *Plasmodium falciparum* in Nigeria. Malaria Research and Treatment. 2013; 13:1-5.
13. Praveen Kumar Bharti, Himanshu Singh Chandel, Amreen Ahmad, Sri Krishna, Venkatachalam Udhayakumar, Neeru Singh. Prevalence of *Pfhrp2* and/or *Pfhrp3* gene deletion in *Plasmodium falciparum* Population in eight highly Endemic States in India. PLOS. 2016;11(8):e0157949.

© 2021 Nassar et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/62106>