South Asian Journal of Parasitology

Volume 6, Issue 4, Page 147-152, 2023; Article no.SAJP.110557

Factors Affecting the Validity of Pfhrp2-Based Rapid Diagnostic Test Kit in the Diagnosis of Malaria in Afikpo, Ebonyi State: A Pilot Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author OTS designed the study, wrote the protocol, performed the statistical analysis, and wrote the first draft of the manuscript. Author ENU and IRK collected the data and managed the literature searches. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/110557

Original Research Article

Received: 12/10/2023
Accepted: 15/12/2023
Published: 20/12/2023

ABSTRACT

Aim: To assess the factors affecting the validity of histidine-rich protein II (HRP2)-based rapid diagnostic test (RDT) kit for diagnosis of malaria in Afikpo North Local Government Area, Ebonyi State.
Study Design: Quantitative methods using both structured questionnaires and serological tests were employed to collect data on factors affecting the validity of HRP2-based RDT kit results for malaria diagnosis.
Place and Duration of Study Sample: Four different health facilities including Akanu Ibiam Federal Polytechnic Medical Centre, Unwana, between November 2022 and March 2023.
Methodology: We interviewed eleven (11) health workers (HWs) (5 males and six females) on factors affecting the validity of RDT kits. We also included 50 patients (19 males and 31 females;

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aged 1-40 years) with symptoms of malaria to validate some of the factors mentioned by the health workers. Replicates of CareStart® TM malaria RDT kits were used to test some of the factors on blood samples collected from the patients. Data collected was analyzed using the Chi-square test.
Results: The majority of the HWs use microscopy (54.5%) while 45.5% use RDT for the diagnosis of malaria. The majority of the HWs claimed that the quantity of buffer reagent (50%) and wait time (14.3%) affects the performance of RDT kits. However, there was no statistically significant difference ($p>0.05$) in the prevalence of malaria (36%) among the different buffer quantities and wait times tested. Hence, the tested buffer reagent volumes and wait times do not affect the performance of rapid diagnostic tests. It is important to validate these findings in future studies with a larger sample size.

Keywords: Malaria; rapid diagnostic test kit; buffer reagent; wait time.

1. INTRODUCTION

Nigeria accounts for the highest proportion (27%) of global malaria cases [1]. Pregnant women and children under five years old are most affected. Most malaria-related deaths happen within a few days from the time an individual starts showing symptoms, and when early diagnosis and prompt treatment with an effective antimalarial drug are delayed. Hence, prompt, and accurate laboratory diagnosis is important for both rapid and effective management and surveillance of malaria [2]. Diagnosis of malaria using light microscopy and polymerase chain reaction (PCR) methods are highly recommended due to their high sensitivity and specificity, but specific challenges have limited their use in malaria-endemic countries like Nigeria. Fortunately, the introduction of rapid diagnostic test (RDT) devices has made these challenges surmountable [3-6].

A malaria rapid diagnostic test (mRDT) kit is a device used to confirm malaria infection in patients presenting symptoms. It detects specific Plasmodium antigens in a small quantity of fresh blood using lateral flow immunochromatography [7]. The types of mRDT in use can detect one or combination of the following antigens: (1) Histidine-rich protein 2 (HRP2)- a water-soluble protein -produced by trophozoites and gametocytes of P. falciparum only; (2) Plasmodium lactate dehydrogenase (pLDH) enzyme produced by all Plasmodium species infective to human; and (3) Plasmodium aldolase enzyme produced by all species of human Plasmodium parasites [8].

Although, mRDT is recommended for malaria diagnosis in resource-limited areas that are endemic for malaria, studies have reported varying levels of its usage and adherence to test results among health workers (HWs) [9]. Reports had shown that RDT was not used by health workers in Enugu state because of the pervasive notion that RDT results were inaccurate [10]. A previous study also showed that 37.6% of health workers in Ebonyi State had a poor perception of malaria RDT kits [9]. Low uptake and confidence in rapid diagnostic test kits may result in inappropriate prescription of antimalarial drugs, economic waste and delayed detection of the primary cause of malaria-like symptoms.

Hence, this pilot study is aimed at assessing factors affecting the validity of RDT kit results in the diagnosis of malaria in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Afikpo under the Afikpo North Local Government Area (L.G.A.) of Ebonyi State, Nigeria. Ebonyi state has the highest prevalence of malaria in southeast Nigeria [11] and malaria is the most prevalent medical condition treated in healthcare facilities across the state [12, 9].

Afikpo is located between latitudes 5° 4′ N and 6° 3′ N and longitudes 7° 5′ E and 7° 55′E. There are predominantly two main seasons: the rainy season between April – October and the dry season between November – March. The annual rainfall is about 160mm – 220mm with maximum precipitation occurring between July and September. The atmospheric temperature is between 23.4°C and 29.9°C and relative humidity is between 60 – 80%. Afikpo is bounded in the North by Ohaozara L.G.A., Ebonyi State, in the East by Afikpo South L.G.A., in the South by Cross River State, and the West by Abia State. The population of people in Afikpo is about 672,000 persons [13-15].

2.2 Administration of Questionnaires

Well-structured questionnaires were administered to medical laboratory scientists (MLS) working in hospitals and primary health centres within three communities (Afikpo, Enohia, and Unwana) within Afikpo North L.G.A. The questionnaires contained questions for collecting data on sex, age, level of education, marital status, knowledge of causes of malaria, mode of malaria transmission, diagnosis of malaria, and perception of factors affecting the validity of RDT kits.

2.3 Sampling of Malaria Symptomatic Patients

Blood samples were collected from 50 malaria patients at the Polytechnic Medical Centre. The blood samples were those for malaria by the medical lab scientist at the Polytechnic Medical Centre.

2.4 Procedure for Malaria Rapid Diagnostic Test

The expiry date of the packet was checked, and the gloves were worn. The packet was opened. Three cassettes were placed on a clean flat surface and labelled clearly with the patient's ID number. The loop was used to put the drop of blood into the square hole marked "S" and the loop was discarded. Two drops of buffer were put into the round hole marked "A", for low buffer volume 1 drop of buffer was put, and for high buffer volume 4 drops of buffer was added. After the buffer was added, we waited for 20 minutes to read the test results (according to the manufacturer's instructions), and then the results were recorded.

2.5 How the RDT Result Was Interpreted

i. One red line in the window "C" and one red line in the window "T" means that the patient was positive for malaria.
ii. One red line in window "C" and no line in window "I" means that the patient was negative for malaria.
iii. No line in the window "C" means the test was invalid.
iv. A window line "T" and no line in the window "C" also means the test was damaged. The results were invalid. Another test was conducted if the result was invalid.

2.6 Testing the Effect of Varying Buffer Volume and Wait Time on the Accuracy of Malaria Rdt Tests

Carestart™ RDT was used for this study.

3 replicates of RDT were used to conduct tests on each blood sample as shown below.
1 RDT for low buffer volume with 1 drop
1 RDT for high buffer volume with 4 drops
1 RDT for recommended buffer volume (2 drops) by the manufacturer (control).
The same procedure was followed for the various wait times tested: low (10 minutes), high (40 minutes), and recommended (20 minutes).

The results of the tests were recorded and analyzed.

2.7 Data Analysis

Data collected was entered in SPSS software version 16.0 for analysis. The chi-square test and Fisher's test were used to determine the effect of buffer volume on the accuracy of malaria test results. The result was presented using percentages in standard tables.

3. RESULTS AND DISCUSSION

3.1 Demographic Characteristics of Health Workers (HWs) Interviewed

The data presented in Table 1 indicates that 45.5% of the medical laboratory scientists (MLSs) who were interviewed were male while 54.5% were female. Most of the MLSs (72.7%) fall within the age range of 31-40 years and all of them have completed tertiary education (100%). A majority of them (63.3%) have work experience of 6-10 years and work in hospitals (45.5%).

3.2 Demographic Characteristics of Malaria Symptomatic Patients

A higher proportion (62%) of patients diagnosed for malaria were female while 38% were male. In terms of their age, most of the patients (64%) are within the age bracket of 21-30 years (Table 2)

3.3 Usage and factors affecting the validity of RDT according to the health workers (HWs)

Results in Table 3 indicate that 54.5% of MLS use microscopy while 45.5% use RDT to

diagnose malaria in their laboratories. Concerning whether RDT is more accurate than microscopy, the majority (81.8%) responded "no" while 18.2% said "yes". Concerning the factors that may affect RDT results, 54.5% believed that buffer quantity can affect RDT results while others mentioned wait time (9.1%), wrong placement of buffer and blood (9.1%), climatic conditions (7.1%), and expired RDT kit (7.1%). However, 18.2% have no idea of any factor that can affect RDT results.

Table 1. Demographic characteristics of health workers (HWs) interviewed

	n(%)
Sex	
Male	5 (45.5)
Female	6 (54.5)
Age (years)	
20-30	1 (9.1)
31-40	8 (72.7)
41-50	1 (9.1)
>50	1 (9.1)
Level of education	
Secondary	0 (0)
Tertiary	11 (100)
Years of work experience	
1-5	1 (9.1)
6-10	7 (63.6)
11-15	1 (9.1)
16-20	0 (0)
>20	2 (18.2)
Type of healthcare	
Primary Health Centre	4 (36.4)
Medical Centre	1 (9.1)
Hospital	5 (45.5)
Private Medical Lab.	1 (9.1)
Total	11

3.4 Effect of Buffer Quantity and Wait Time on RDT Result

There was no significant difference (p=1) in the number of samples that tested positive and negative within the various test groups. Out of the 50 samples analyzed, 36.0% were positive to Plasmodium falciparum while 64.0% were negative. Concerning quantities of buffer volumes tested, the prevalence of malaria (36%) was the same at low, normal, and high buffer volumes respectively. Concerning the various wait times tested, the prevalence of malaria (36%) was also the same at low, normal, and high wait times respectively.

Table 2. Demographic characteristics of patients diagnosed for malaria

	n(%)
Sex	
Male	19 (38)
Female	31 (62)
Total	50 (100)
Age (years)	
0-10	5 (10)
11-20	8 (16)
21-30	32 (64)
31-40	5 (10)
Total	50 (100)

Table 3. Health workers' usage and factors affecting the validity of RDT kits

	n(%)
How MLS diagnose malaria in their lab.	
Use RDT	5 (45.5)
Use microscopy	6 (54.5)
Total	11
Is RDT more accurate than microscopy?	
Yes	2 (18.2)
No	9 (81.8)
Total	11
Perceived factors that affect RDT result	
Wait time	2 (14.3)
Buffer quantity	7 (50.0)
Wrong placement of buffer and blood	1 (7.1)
Climatic conditions	1(7.1)
Expired RDT	1(7.1)
No idea	2 (14.3)
Total	14

3. DISCUSSION

The accuracy and validity of malaria rapid diagnostic test (RDT) kits can be influenced by various factors, and temperature, humidity and RDT preparation are among such factors [16]. The findings from this study showed that the majority of the health workers had a wrong perception of factors that can affect the validity of RDT kits in the diagnosis of malaria. There is no valid report that reagent (buffer) volume and wait time affected the performance of RDT kits. Going a step further in this study, we reported that the various volumes of buffer solution and wait time assessed did not affect the validity of RDT kit performance.

Table 4. Effect of buffer quantity and wait time on validity of RDT kits used for diagnosis of malaria in symptomatic patients

Test groups	RDT result n(%)		Total	Fisher's test
	Positive	Negative		
Quantity of buffer				
Low	18 (36.0)	32 (64.0)	50 (100)	Z=0
Normal (recommended)	18 (36.0)	32 (64.0)	50 (100)	P=1
High	18 (36.0)	32 (64.0)	50 (100)	
Wait time				
Low	18 (36.0)	32 (64.0)	50 (100)	
Normal (recommended)	18 (36.0)	32 (64.0)	50 (100)	
High	18 (36.0)	32 (64.0)	50 (100)	

It is interesting to note that the use of inappropriate buffer reagents can affect the performance of RDT kits. Buffer reagents play a crucial role in ensuring the proper functioning of RDTs by facilitating the reaction between the sample and the test components. In the case of malaria RDTs, the buffer solution is designed to optimize the conditions for the interaction between the target antigens in the patient's blood and the antibodies or other detection molecules on the test strip. Substitution of buffer reagents can affect the performance of RDT kits in several ways including its sensitivity and specificity, reaction rate on the test strip, interference with test components, and storage stability [17, 18]. However, in the present study, substitution of buffer reagent was not investigated.

Hence, the use of buffer reagents recommended by the RDT kit manufacturer is encouraged to ensure optimal performance. The present does not in any way encourage RDT kit users to deviate from manufacturers' specific instructions on the preparation and use of the buffer solution. It is recommended that users of RDT kits refer to the specific product documentation and guidelines provided by the RDT kit manufacturer for accurate information on buffer reagents and test procedures.

4. CONCLUSIONS

The various buffer reagent volumes and wait times tested in the diagnosis of malaria using an RDT device did not affect the validity of the RDT kits.

ACKNOWLEDGEMENTS

We acknowledge the role of data collectors who assisted in administering questionnaires to the health workers. We also appreciate the patients that volunteered to participate in the study and the medical laboratory scientists who collected blood samples from them. We thank the host institution that provided the enabling environment for the study to be conducted. This study was self-funded; hence, we acknowledge the co-authors for providing funds to conduct the study.

CONSENT

All authors declare that written consent was obtained from the patients that participated in the study before sample collection.

ETHICAL APPROVAL

The study proposal was reviewed by the biology research unit in the Science Laboratory Technology department, A.I.F.P.U., Ebonyi State. Approval to conduct the study was also obtained at the Medical Centre of the host institution

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/110557