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COMPARATIVE EVALUATION OF TWO RAPID MALARIA DIAGNOSTIC TESTS KITS AMONG HEALTH-CARE SEEKING INDIVIDUALS IN ILORIN, NORTH CENTRAL NIGERIA

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Highlights of the study

- The study compared both the microscopic and two rapid diagnostic test kit methods used in the diagnosis of malaria in southwestern Nigeria
- Nova test kit perform better than CareStart^{TM} test kits
- The positive and negative predictive values for the test kits were not significantly different

ABSTRACT

Background: Malaria microscopy remains the gold standard for the diagnosis of malaria. However, lack of laboratory consumables, standard microscopes and expertise in malaria microscopy, especially in the rural areas with high malaria transmission, limits its deployment. Rapid Diagnostic tests (RDT) which are alternatives to the gold standard must however be evaluated in comparison with the microscopy before massive deployment of such RDT product in the field. This study evaluated two RDT products (CareStart^{TM} and Nova) using blood sample of symptomatic and asymptomatic health care seeking individual in a health care center in Ilorin. Capacities of the products were compared with the microscopy in the diagnosis of malaria.

Methods: A total of 122 blood samples were collected through venipuncture of post-cubical veins of the patients using a syringe. The blood samples collected from each patient were transferred to a clean pre-labeled bottle containing ethylenediaminetetra acetic acid (EDTA). Two RDT products: CareStart^{TM} *Pf* and Nova *Pf*/*Pv* were purchased for evaluation or comparison with microscopy.

Results: Overall, 122 health care seeking individuals were examined. The sensitivity and specificity for *Plasmodium falciparum* malaria were 47% and 82%, respectively for CareStart^{TM} RDT while 52% and 83% were for Nova RDT respectively. There was no significant difference ($p>0.05$) in the sensitivity and specificity between the two RDTs. The positive and negative predictive values of the two test kits were not significantly different: CareStart^{TM} (87% and 37%), Nova (90% and 36%). The Nova RDT however, performed better than the Carestart^{TM} though with a close margin.

Conclusion: The two RDT products could be used as the first screening test for malaria diagnosis thereby improving on the current situation of treating patients for malaria without laboratory outcome to confirm clinical evaluation.

KEYWORDS: Malaria, Microscopy, Rapid Diagnostic Test

INTRODUCTION

Malaria is still a deadly disease with 241 million cases and 627, 000 deaths worldwide in 2020 [1]. The global burden is dominated by countries in sub-Saharan Africa: Nigeria has the highest number of malaria cases (27%), followed by the Democratic Republic of Congo (12%) [1]. In Nigeria, malaria accounts for 60% of outpatients and 30% of hospitalizations in children under the age of 5 years. An estimated value of 300,000 deaths due to malaria infection is recorded in Nigeria every year [2]. Following massive enlightenment of people as regards the resistance of the *Plasmodium* parasite to older antimalaria drugs, there has been an increase in the use of artemisinin-based combination therapy (ACT) drugs as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria [3]. While *Plasmodium* parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly confirmed, resulting in over-use of anti-malarial drugs and poor disease monitoring [4].

The use of Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS), prompt diagnosis and adequate management of uncomplicated malaria have significantly reduced morbidity and mortality in many endemic areas of Africa [5, 6].

Accurate confirmation of malaria diagnosis can reduce the over use of ACT treatment, thereby, reducing the risk of adverse drug reactions and delay the development of resistance to the drugs [1]. Accurate malaria diagnosis and improved public health data reporting systems are the keys to adequate treatment of malaria [7]. Parasitological confirmation using microscopy or a Rapid Diagnostic Test (RDT) is recommended for patients with suspected malaria before treatment [1].

The accepted gold standard diagnostic method involves microscopic reading of a stained blood film. However, it is usually time consuming, labour intensive and requires well trained and skilled personnel, good quality reagents, and well-maintained microscopes [8]. Ability to obtain microscopic results in less than two hours, as recommended by WHO, is very challenging especially in Africa where stable electricity

supply, experienced personnel, good microscopes and its associated consumables are often unavailable. Poor or outright unavailability of reliable malaria parasite microscopic diagnosis has led to the use of rapid diagnostic techniques at almost every level of health care centers [8].

Rapid diagnostic tests technology is attractive to health care settings lacking reliable microscopic examination expertise especially those facing a high case load of patients with suspected malaria [9]. These tests can be used by personnel including laypersons, and not necessarily laboratory technicians, after limited training. Malaria RDT results are on average available in less than 30 minutes [9].

The RDTs work by detecting specific malaria antigens or enzymes and are designed using antibodies against parasite species-specific or genus-specific antigens [10]. Histidine-rich protein II (HRP2) and lactate dehydrogenases (pLDH) are some of the proteins detected by malaria RDT [11]. The HRP2 antigen is one of the three histidine-rich proteins produced solely by trophozoites and young gametocytes of *Plasmodium falciparum*. Therefore, RDTs based on the detection of HRP2 can only diagnose *Plasmodium falciparum* infections and thus cannot be used for the detection of other human malaria [11].

The limitation of HRP2-based tests is their persistent false positive result after effective treatment of the infection, since HRP2 is only slowly eliminated from the blood stream as it is expressed in the erythrocyte membrane. This fact renders inconclusive a positive RDT result with history of a recently treated infection, especially in areas of high transmission [11]. All of these raise reliability issues for RDTs as means of malaria diagnosis compared to the generally accepted microscopy method. Yet there is a wide variety of RDTs available in the market presently and their different performance under various endemic settings suggest that careful comparative assessments of RDTs are required before mass deployment for diagnosis. This study therefore investigates the performance of two different RDT products in the diagnosis of malaria infection among health care seeking individuals in Ilorin, Kwara State, Nigeria.

MATERIALS AND METHODS

Study Area and Population

The study was conducted in a private health care centre in Ilorin metropolis, Kwara state. Kwara state is situated in the North Central zone of Nigeria. The human population in Ilorin which was 780,771 in 2005 [12] has been projected to reach 3,518,771 people in 2020 [13]. The climate is tropical with mean annual temperature, relative humidity and rainfall of 25-28.9oC, 65-80% and 1150 mm, respectively [14].

Sample Collection and Analysis

A total of 122 blood samples were collected through venipuncture of post-cubical veins of the patients using a syringe. The blood samples collected from each patient were transferred to a clean pre-labeled bottle containing ethylenediaminetetra acetic acid (EDTA). These blood samples were used to prepare thick and thin smears on glass slides and for testing with the two different RDT kits. Persons included in the study were individuals attending the out-patient section of the hospital, pregnant woman, adults and children were included. Two RDT products: CareStart^{TM} *Pf* and Nova *Pf*/*Pv* were purchased for evaluation

or comparison with microscopy. A drop of blood was placed in the blood well of each RDT product and followed by a drop of buffer. Manufacturers' guidelines were strictly followed for each of the RDT product.

Thick and Thin Blood Smear Preparation

Preparation of thin and thick blood smears and examination of slides under the microscope was conducted following the WHO protocol [15]. The slides were air-dried, and the thin film fixed with methanol, both films were stained with giemsa stain. The stained slides were rinsed under mild running tap water and allowed to air-dry before microscopic examination under x100 oil immersion lens.

Data Analysis

To compare the performance of the two RDT products with microscopy, sensitivity, specificity, Negative Predictive Values (NPV) and Positive Predictive Values (PPV) were calculated [16]. Performance indices were calculated for malaria as a whole and not according to species of *Plasmodium.* The variables measured were number of true positives (TP), number of true negatives (TN), number of false positives (FP) and number of false negatives (FN). Results were considered false positive, if *Plasmodium* parasite were detected in RDT (CareStart^{TM} or Nova) and could not be detected on thick smear and vice versa.

RESULTS

Malaria prevalence among study population

A total of one hundred and twenty-two individuals comprising those who visited the hospital for malaria treatment (99) and those who visited for the treatment of other ailments (23) were studied. Seventy five percent of the malaria treatment seekers were found to be infected while 56% of those who visited the hospital for the treatment of other ailments were also positive according to the blood films examined under the microscope. From the total number of blood films found to be malaria positive (88) and the overall number of blood films examined, the total malaria positive rate which represents the malaria prevalence in this study was 72% (Table 1).

Table 1: Malaria prevalence among residents in Ilorin

Ailment groups	Blood film examined	Malaria positive	Slide positive rate (%)
Malaria treatment seekers	99	75	76
others	23	13	57
Total	122	88	72

Fourty one individuals were found to be malaria positive (True positive) after both tests (microscopy and RDT using CareStart^{TM}) were conducted while 28 others were negative (True Negative). On the other hand, 47 healthcare seekers were positive to microscopic blood film examination but negative to the CareStart^{TM} RDT analysis (False Negative) while 6 others were found to be negative to microscopic

examination but positive to CareStart™ RDT anlaysis (False Positive). The CareStart™ RDT test was sensitive (47%) and specific (82%) for the diagnosis of *P. falciparum* with a PPV and an NPV of 87% and 37%, respectively (Table 2).

Table 2: Results of CareStart™ and thick blood films of the residents.

RDT	Positive thick blood film	Negative thick blood film	Total
Positive CareStart™	41	6	47
Negative CareStart™	47	28	75
Total	**88**	**34**	**122**

Sensitivity = 41/88×100 = 47%, Specificity = 28/34×100 = 82%, PPV = 41/47×100 = 87%, NPV = 28/75×100 = 37%

For Nova RDT, 48 individuals were found to be malaria positive (True Positive) after both tests (microscopy and Nova RDT) were conducted while 25 others were negative to both tests (True Negative). On the other hand, 44 health care seeking individuals were positive to microscopic blood film examination but negative to the Nova RDT analysis (False Negative) while 5 others were found to be negative to microscopic examination but positive to Nova RDT analysis (False Positive). The Nova RDT was sensitive (52%) and specific (83%) for the diagnosis of *P. falciparum* with a PPV and an NPV of 90% and 36%, respectively (Table 3).

Table 3: Results of Nova and thick blood films of the residents

RDT	Positive thick blood film	Negative thick blood film	Total
Positive Nova	48	5	53
Negative Nova	44	25	69
Total	**92**	**30**	**122**

Sensitivity = 48/92×100 = 52%, Specificity = 25/30×100 = 83%, PPV = 48/53×100 = 90%, NPV = 25/69×100 = 36%

DISCUSSION

The results from this study show the performance of two different RDT products available in the Nigerian market, compared to the gold standard method. The overall malaria prevalence rate in this study was 72%. This prevalence rate confirmed that malaria transmission is still high in this part of the country. The 72% prevalence rate was lower compared to earlier report of 79.4% recorded in Ilorin [17] probably because of the focus of the latter on pregnant women who are more susceptible to the malaria parasites. However, the 72% prevalence rate recorded in this study was higher compared to other reports of 55% recorded in Ibadan, Southwestern Nigeria [18] and 50% in Jos, Northcentral [19].

The sensitivity obtained in this study for Nova (52%) was higher compared to CareStart™ (47%) probably because Nova RDT can detect both *P. falciparum* and *P. vivax* as against CareStart™ which can only detect *P. falciparum*. However, the PPV (carestart™ 87%, Nova 90%) and NPV (Carestart™ 37%, Nova

36%) values for both RDT products were similar.

Considering that appropriate malaria RDT should have high sensitivity (95%) and specificity (97%), and ability to detect low parasite density infections [20], the two sensitivity values (Carestart^{TM} 47%, Nova 52%) obtained in this study were still very low.

In this study, the Carestart^{TM} *P.f* (47%) and Nova *P.f /P.v* (52%) RDT sensitivities were low while specificities were found to be higher (82% and 83% respectively) for *P. falciparum* diagnosis. This finding is similar to the report of 58.8% SD Bioline's *P.f /P.v* RDT sensitivity elsewhere [21]. However, the reported PPV and NPV of 58 and 68% were at variance with the PPV (carestart^{TM} 87%, Nova 90%) and NPV (Carestart^{TM} 37%, Nova 36%) obtained in this study. Although, parasite density was not considered in this study, these differences could be as a result of differential parasitemia density since the quantity of antigen *Pf/Pv* detected by the RDTs is in direct proportion to the number of parasites in the blood (Markler and Hinrichs, 1993). In addition, it was noted that SD bioline Pf/Pv RDT sensitivity was 100% when the parasitemia was higher than 1000uL but decreased with lower parasitemia levels [22].

The specificity value (42.9%) obtained in another report [21] was also lower than this study (CareStart 82%, Nova 83%). However, the specificity value obtained in this study is still lower than the recommended 97% [20]; this could be due to self-treatment which is common among health care seekers in urban Nigerian localities like our study area. The self-treatment might have cleared the parasitemia but have residual circulating HRP-2 antigens [23].

CONCLUSION: Overall, Nova RDT performed better than CareStart^{TM} according to the results of this study though the sensitivity and specificity values of careStart^{TM} were very close to Nova. Both products could serve as useful epidemiological tools especially as first screening tests in the diagnosis of *Plasmodium falciparum* malaria in Ilorin, Kwara State, Nigeria.

Highlights of discussion

- The study compared both the microscopic and two rapid diagnostic test kit methods used in the diagnosis of malaria in southwestern Nigeria
- Nova test kit perform better than CareStart^{TM} test kits
- The positive and negative predictive values for the test kits were not significantly different

Established Facts

- Malaria is a public health burden in Nigeria
- The cost of management is relatively high and it kills both young and old patients
- The diagnosis of malaria is one of challenges faced during visit to public health centres because the time spend before the result of malaria microscopy is too long
- Rapid diagnostic method remains an alternative to malaria microscopy

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Ethics consideration/Statement of Ethics:
Ethical approval was obtained from individual attending the public health centre before the commencement of this work

Ethical Review Board:
We received ethical approval from the ethical review committee from Kwara State University, Malete, Kwara State, Nigeria

Informed consent
Prior to the commencement of this study, informed consent was obtained from all participants

Conflict of Interest Statement
The authors have declared that there are no conflicts of interest

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Authors Contribution
Abiodun Obembe[1], Opeyemi G. Oso[2] and Shola K. Babatunde[3]
Author 1 and author 2 conceived the experiment and prepared the manuscript; author 1 and author 3 analyzed the data. All authors approved the final manuscript.

Data Availability Statement
All data were provided in the manuscript

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