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Performance of Rapid Diagnosis Technique (RDT) in Screening Malaria Among Patients of Selected Health Facilities in Ardo-Kola Local Government Area, Taraba State

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Abstract: Malaria infection is one of the most common disease of public health importance afflicting millions of people in sub-Saharan Africa. Malaria diagnosis and surveillance rely predominantly on rapid diagnostic tests (RDTs). This study investigated the performance of Rapid Diagnostic Test (RDT) against microscopy of stained blood for *Plasmodium falciparum* of patients attending some Primary Health Centres in Ardo-Kola LGA, Taraba State. Five hundred and eighty five (585) blood samples were collected and examined for *Plasmodium falciparum* on the microscope. Rapid Diagnostic Tests were also used for examination. The overall result showed a prevalence of 446 (76.23%). The infection found female, 199 (82.23%) to be more exposed to malaria than male, 247 (72.01%) with no significant difference (χ^2=4.381; P≥0.05). With regards to age, females aged ≥51 years were more infected than other age groups, while male aged 21-30 years were more infected with malaria than the other age groups. There was no significant difference with malaria infection between the age groups (χ^2=2.207; P≥0.05). The performance of RDTs against microscopy showed that RDT used had a sensitivity of 66.91% and specificity of 58.52%. The positive predictive value of 33.45% and negative predictive value of 85.02% were found for the RDT used. The present study demonstrated that RDTs can act as diagnostic tool to manage malaria in resource poor settings with limited, access to expert microscopy as they are easy to use and perform better than microscopy. It is recommended that malaria Rapid Diagnosis Tests can be used in endemic areas in Nigeria.

Keywords: Rapid, Diagnostic, Test, Sensitivity, Specificity, Positive

1. Introduction

Malaria is one of the most important public health problems in terms of morbidity and mortality, causing more than 200 million cases and 445,000 deaths every year [1]. A total of 216 million estimated malaria cases occurred in 2010, 81% of which were reported in the Africa region (5%) the number of malaria deaths was estimated to be 445,000 in 2017; 91% of whom occurred in the African region, 6% in South-East Asia and 3% in eastern Mediterranean Region although the proportion exposed to malaria parasites has decreased during the last century, the absolute number of people at risk for malaria infection increased from 0.8 billion in 1900 to 3.3 billion in 2017, as a consequence of the

absolute increase of the population living in malaria mortality rates showed a global reduction of 25% between in 2017 [1-3].

Malaria in humans is caused by five (5) *Plasmodium* parasites: *Plasmodium falciparum, P. malariae*, *P. ovale* and *P. knowlesi*. The current distribution of human-pathogenic *Plasmodium* species shows preponderance of *P. falciparum* in tropical Africa, while *P. vivax* prevails over *P. falciparum* in South America. Both *P. falciparum, P. vivax* and *P. knowlesi* are prevalent in south-easthern Asia and western pacific. Although *P. malaria* may occur in all malaria area, its prevalence is generally low. In tropical Africa, *P. falciparum* and *P. malariae* co-infection is sometimes encountered. *P. ovale* is widespread principally in tropical Africa [1].

Malaria parasites are endemic in some regions where there is fairly constant number of cases throughout the year, year, while region have "malaria season" mostly during rainy season. Malaria transmission is intense all year round in the forest belt but in the dry savannah, transmission is relatively low during the dry season [2-5]. The factors that contribute to the spread and transmission of malaria depend on the interaction between the human host, anopheles vector, malaria parasite and environmental conditions [6, 7].

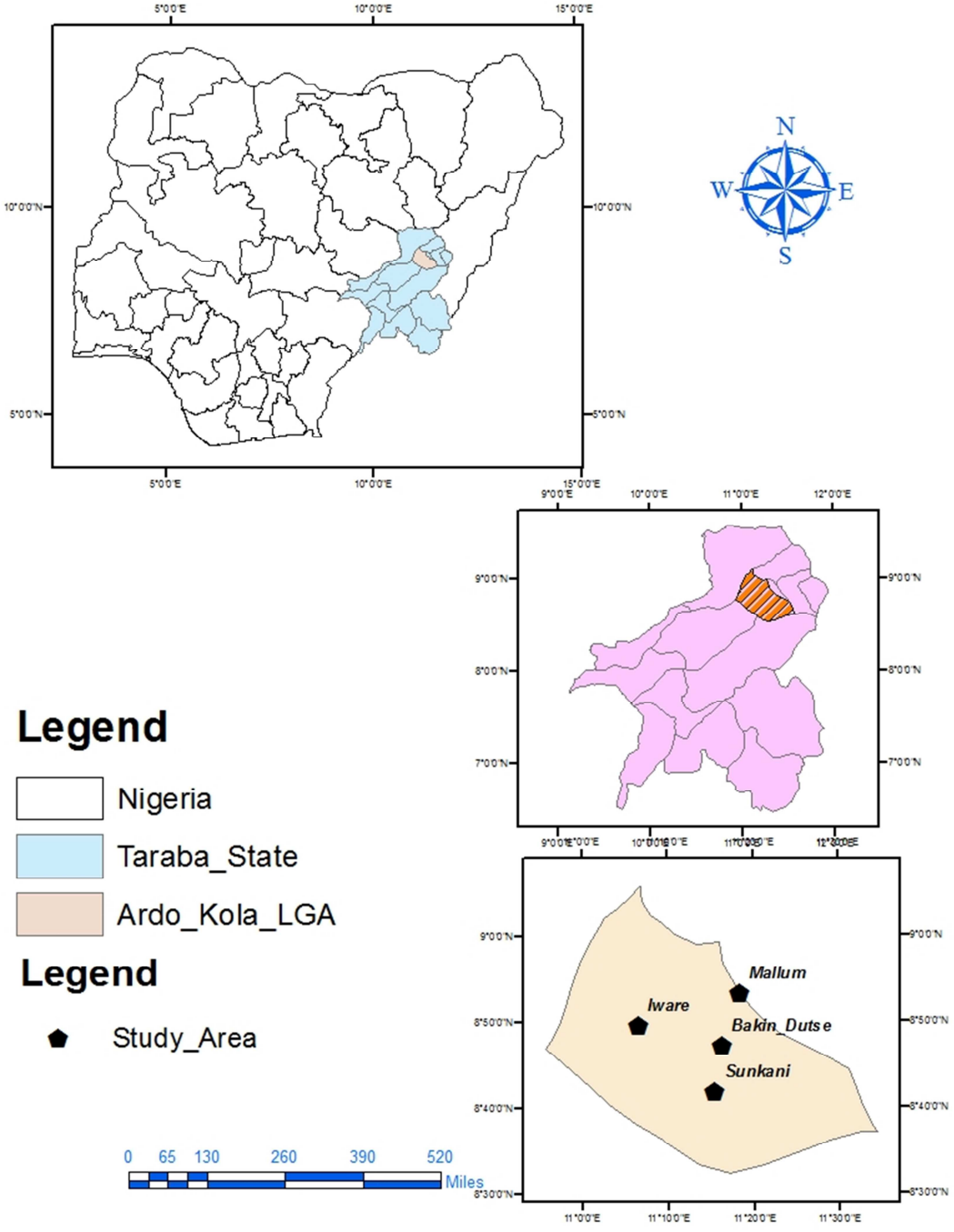

Figure 1. *Map of study area.*

The *Plasmodium* species responsible for malaria in Nigeria are *Plasmodium falciparum*, *P. malariae* and *P. ovale* [2]. In his study, it was shown that more than 80% of malaria infection were caused by *Plasmodium falciparum,* while up to 15% were caused by *P. malariae* and less than 5% by *P. ovale* furthermore, mixed infection with *P. falciparum* has been reported in Nigeria. Nigeria holds one of the largest shares of the world's burden of malaria [8].

The overwhelming burden of malaria raises the need for extensive research on the impact of malaria on health outcomes. Hence, this research was conducted to evaluate the sensitivity and specificity of a rapid diagnostic technique used and its comparison to the gold standard microscopy.

2. Materials and Methods

2.1. Study Area

Ardo-kola is a Local Government Area in Taraba State, which is in the Northeast geo-political zone of Nigeria (Figure 1). The local government area has an estimated population of 86, 921 from the 2006 National census. It has borders with Gassol and Bali LGAs in the south, Jalingo LGA in the North, Yorro LGA to the East. The districts within Ardo-kola are: Alimgora, Ardo-kola, Iware, JauroYinu, Lamido Borno, Mayo Renewo, Sarkin Dutse, Sunkani, Tau and Zangon Kombi. The Local Government lies between longitude 9° 17" N and latitude 9° 59"E (Figure 1). The residents of the local government predominately speak Jukun Kona, Mumuye, Fulani and Hausa languages among others and they are mostly farmers. The climate in the area can be described as tropical sub- humid type with two distinct (wet and dry seasons) It has an average rainfall of 7 months annually with total range between

1,200mm and 2000mm in the months of April and October. The temperature is relatively high throughout the year averaging 28°C-36°C with an occasional peak at 44.0°C between March and April.

2.2. Study Population

A total of 585 subjects clinically diagnosed of malaria were randomly selected from four health facilities in Ardo-kola Local Government Area of Taraba State for the study.

2.3. Ethical Permission

Ethical permission was sought and obtained from Postgraduate Review Committee of Biological Sciences Department, Taraba State University. Furthermore, additional permission was sought and obtained from the Director, Primary Health Care (PHC), Ardo-kola Local Government of Taraba State before the commencement of study.

2.4. Study Design and Sample Size Determination

The research design for this study is cross-sectional descriptive study. Prior to commencement of sample collection, oral consent of participants recruited for the study were sought and obtained. Furthermore, questionnaires which sought on locality, age, sex, occupation and educational status was administered to participants. The selected persons were assured of utmost confidentiality before collection of blood sample. A sample size of 585 was used based on the formula described by Fisher (1998) for proportions in a population greater than 10,000.

$N=Z_2pq/d^2$, where N=sample size, Z=2.58, p=prevalence, q=1-p, d=0.01. The calculated population size per health centre is 142. The minimum population to be considered is then 142 x 4=568.

2.5. Procedure for Data Collection

Blood samples were collected from patients visiting primary health care facilities and other private hospitals around Ardo-kola Local Government Area, Taraba State. Specimen from patients, regardless of their age and sex who have not received any anti-malarial drugs for the past two months with clinical presentation of malaria such as fever, headache, rigors, vomiting, diarrhoea, general malaise, weakness enlarged spleen and liver among subjects were used for the study.

2.6. Screening of Blood Samples

Blood samples withdrawn from patients were collected inside sample bottles containing anticoagulant ethylene diaminetetracetic acid (EDTA). Thorough mixing of the blood with the anticoagulant was done to avoid coagulation. Thin blood films were made from the blood samples, fixed with methanol and stained with Giemsa stain and examined under the microscope using the oil immersion objective. A rough estimate of parasitaemia was made for specimen selection. Blood samples with films showing large ring stage of *P. falciparurn* parasites were processed for other haematological examinations [9].

2.7. Preparation of Thin Blood Films

A drop of blood was placed on a clean grease free slide, about 1 cm from one end, and a spreader with smooth edge was placed in front of the drop of blood inclined at angle of 45° was placed in front of the drop of blood. This spreader was drown backward to make contact with the drop of blood. Afterwards, a quick forward movement was made to enable the blood spread out. The thin film was made to cover about half of the slide and to assume tongue shape. The thin film was air-dried and labelled accordingly [9].

2.8. Preparation of Thick Films

Thick and thin blood films were prepared on the same slide for each subject and stained using 10% Giemsa as described by Cheesbrough [9]. Using a completely clean grease free microscope slide a small drop of blood was added to the centre of the slide and a large drop about 15mm to the

one end of the slide. Using a smooth edge slide spreader, the thin blood film was spread immediately without delay, the large drop was spread to make a thick film to cover an area of about l5 x l5mm. The slide was labelled with a 1 lead pencil, indicating date, patient name and number. The film was allowed to air dry. The thin film was fixed with absolute methanol for three minutes without touching the thick films.

2.9. Rapid Diagnostic Test

Inside the cassette is a strip made of filter paper and nitrocellulose. Typically, a drop of blood is added to the RDT through one hole (A, sample well) and then a number of drops of buffer usually through another hole (B, buffer well). Buffer carries the blood along the length of the RDT. The first step of the test procedure involves mixing the patient's blood with a lysing agent in a test strip or well. After the drop of blood and buffer on the strip, the appearance of blue line showed the negativity of malaria. The appearance of blue line and red line showed the positivity of malaria in infected individuals. When there is no appearance of lines there was an invalidity of the test used.

2.10. Statistical Analysis

Performance of the Rapid Diagnostic Tests was assessed by the calculation of Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) using formulae. The significance of the analysis was $p \leq 0.05$ at 95% of the confidence interval.

$Sensitivity = \frac{a}{a+b}$, where a=True Positive, b=False Positive

$Specificity = \frac{c}{c+d}$, where c=True Negative d=False Negative

$PPV = \frac{a}{a+d}$, where a=True Positive, d=False Negative

$NPV = \frac{c}{c+b}$, where c=True Negative, d=False Negative

3. Results

A total of 585 patients were examined for malaria parasite. Of the number, 446 (76.23%) were infected (Table 1).

Table 1. *Prevalence of P. falciparum based on gender and age groups among patients attending Ardo-Kola Primary Health Care facilities in Taraba State, Nigeria.*

Age	Male		Female		Total	
	No. exam	No. inf (%)	No. exam	No. inf (%)	No. exam	No. inf (%)
1-10	93	70 (71.42)	77	62 (80.51)	170	132 (22.56)
11-20	37	17 (45.94)	39	35 (89.74)	76	52 (68.42)
21-30	26	23 (88.46)	15	13 (86.66)	41	36 (87.80)
31-40	41	26 (63.41)	17	15 (88.23)	58	41 (70.68)
41-50	76	51 (67.10)	72	53 (73.61)	148	104 (70.27)
≥ 51	70	60 (85.71)	22	21 (95.45)	92	81 (88.04)
Total	343	247 (72.01)	242	199 (82.23)	585	446 (76.23)

Chi-square for *P. falciparum* in sex (χ^2=4.381; P≥0.05)
Chi-square for *P. falciparum* in age (χ^2=2.207; P≥0.05)

Table 2 compares the use of RDT as a rapid test to the true disease status as determined by microscopy in the study area. In all *P. falciparum* was detected by RDT in 307 persons. Out of these, 261 (85.01%) persons were positive for both RDT and microscopy (true positive) and 46 (14.98%) persons with antigenaemia but were not detected by microscopy (false negative). Of the 278 subjects negative for RDT, 185 (66.54%) were positive for microscopy (false positive) while 93 (33.45%) were negative for microscopy (True negative).

Table 2. *Comparison of Rapid Test and Standard Microscopy and Rapid Diagnostic Test.*

Microscopy	Negative	Positive	Total
Negative RDT	93[c] (33.45)	185[b] (66.54)	278 (47.52)
Positive	46[d] (14.98)	261[a] (85.01)	307 (87.21)
Total	139 (23.76)	446 (76.23)	585

Keys:
a=True Positive
b=False Positive
c=True Negative
d=False Negative

Table 3 shows the validation of RDT as screening test for *P. falciparum* infection in the study area. The ability of the RDT to accurately identify all those with the disease (the (sensitivity) was 66.91% with confidence interval (54.15%-78.42%) while the ability of the RDT to sort out all those without the disease (specificity) was 58.52% with confidence interval (48.52%-67.49%) The Positive Predictive value was 33.45% [CI: 23.68%-45.05%] while the Negative Predictive Value was 85.02% [CI: 76.23-91.58%].

Table 3. *Sensitivity, Specificity and Predictive Values of Rapid Diagnostic Test.*

Parameters	Performance (%)	Confidence Interval (CI)
Sensitivity	66.91	54.15%-78.42%
Specificity	58.52	48.52%-67.49%
Positive Predictive Value	33.45	23.68%-45.03%
Negative Predictive Value	85.02	76.23%-91.58%

4. Discussion

In this study also, routine microscopic examination of stained blood films was considered as the gold standard for malaria diagnosis. The RDT had Sensitivity of 66.91% with a specificity of 58.52%. The relative good sensitivity has a

possibility of easy examination of malaria which is a good advantage in rural areas. The sensitivity of rapid diagnostic test at level of parasitaemia and for non-immune population remains a problem. compared to stained blood film microscopy. The RDT were found to be less sensitive in detecting asymptomatic patients particularly at low parasitaemia [8, 10, 11]. Furthermore, the rapid diagnostic tests had been reported to have given false negative result even at higher level of parasitaemia [8, 12, 13]. Therefore in case of suspected severe malaria or complex health problem emergences, a negative result may not necessary rule out malaria. It followed that negative RDTs should always be confirmed by microscopy [14, 15].

Concurrently, WHO has begun a dialogue with scientist, clinicians and manufacturers of malaria rapid diagnosis test devices, regarding the realistic possibilities for developing accurate, sensitive and cost effective rapid diagnostic test for malaria diagnosis services. Limitation for those rapid diagnostic tests include incapability for the test to detect 100 parasites/µl for *Plasmodium* species and inability to perform semi-quantitative measurement for manufacturing drug treatment results. For better quality assurance, the new technologies must be compared against "gold standard method [1].

5. Conclusion

Rapid Diagnostic Test with its characteristic is the best hope for diagnosis as a key component of successful malaria. Rapid Diagnostic Technique offers a good alternative, being an easy and rapid method that does not require an experienced laboratory scientist. We recommend that rapid diagnostic test should be used with stained blood film microscopy to ascertain the degree of infection and to know the malaria species involved so as to proffer treatment. World Health Organization should liaise with laboratory scientists, clinicians and manufacturers of malaria rapid diagnostic test, to produce a more accurate rapid diagnostic device that can detect malaria parasites at very low density.

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