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Sensitivity of Rapid Diagnostic Test and Microscopy in Malaria Diagnosis in Iva-Valley Suburb, Enugu

Nwosu Chinemelum Joyce[1*], Ekwunife Chinyelu[2], Nkwocha Elaine Adaku[3]
[1,2]*Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria*
[3]*Department of Microbiology, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Nigeria*

***Abstract*: Malaria disease is still of public health importance. For effective management and control, an alternative parasite-based diagnosis is paramount. This study focused on determining the sensitivity of rapid diagnostic tests and microscopy in malaria diagnosis in Iva-valley suburb, Enugu State, Nigeria. A total of 379 blood samples were collected from five communities and examined for malaria parasites using microscopy and rapid diagnostic test. Out of the 379 blood samples, 166 (43.80%) were positive for malaria parasites using the CareStart test kit, while 169 (44.6%) were positive using microscopy. Using microscopy as the gold standard, the sensitivity and specificity of the CareStart RDT were 89% and 92.86% respectively, while the positive and negative predictive values were 90.96% and 91.55% respectively. Females had the highest prevalence (69.2 %,) while males had the least (30.8%). However, the difference in gender prevalence was not significant (X2 calc = 1.939, X2 tab=3.841, P>0.05). The age group 11-15 years had the highest prevalence (27.8%), while the 21-25 years age group had the least prevalence (1.18%). This study showed that the accuracy of CareStart RDT is comparable to the gold standard microscopy, making it a suitable diagnostic tool for any local health staff in remote endemic areas.**

***Keywords*: Malaria, CareStart RDT and Microscopy**

1. Introduction

Malaria a seasonal disease remains a major public health problem in both tropical and subtropical countries in Africa, major incidence occurs during the rainy season. 1-2 Malaria remains the leading cause of mortality and morbidity in sub-Saharan Africa, 3 an estimated cases of 229 million and 409 000 estimated death in 2019.4 Nigeria is known for high prevalence of malaria and the disease remains one of the leading causes of childhood and maternal morbidity and mortality, low productivity and reduced school attendance in Nigeria [5]. Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood. Diagnosis of malaria infection based on clinical symptoms alone is unreliable because the symptoms of malaria are non-specific and often overlaps with other febrile diseases.6 Lack of precise diagnosis remains an important obstacle to the treatment adherence, effectiveness and clinical managements of severe cases.

For effective management of malaria infection, prompt and accurate diagnosis is very essential.7 The use of antigen-detecting rapid diagnostic test kits (RDTs) forms a vital part of providing parasite-based diagnosis in areas where good quality microscopy cannot be maintained.3 Also Rapid diagnostic test do not require laboratory support, are easily read and can reach sensitivity similar to that commonly achieved by well-performed microscop.8 Other diagnostic methodologies have also risen to overcome the inefficient malaria diagnosis such as Polymerase Chain Reaction (PCR) based genetic tests.

2. Materials and Methods

1) Study Area

Iva valley is a sub-urban settlement in Enugu Metropolis in the capital city of Enugu State of Nigeria. It has geographical co-ordinates of approximately 60.14" and 60.18" North latitude and 70.5oC and 70.09" East longitude. It is located in the tropical rainforest zone, although it has derived savanna vegetation. It has two marked seasons, the dry and wet seasons. There are about 8 months (April – November) of wet season and four months (November – March) of dry season. It has a relative humidity of 70% reaching 80% during rainy season and an annual rainfall of about 2000mm. The daily temperature ranges from 26oC – 35oC during the dry season stretching from November to March, and from 220C –30oC during the wet season stretching from April to November. The inhabitants are mainly Igbos'. The settlers are retired coal miners, civil servants, traders, craftsmen, educated people and farmers. With the creation of Anambra State in 1991, and the establishment of Government machineries' and institutions of higher learning, over 60% of the populations are civil servants and students while the remaining 40% are farmers, traders and other occupations. This structure is still the same when Enugu State was carved out of Anambra State.

2) Community mobilization

A letter of intent to carry out the study at Iva valley was collected from the Head of Department of Parasitology and Entomology addressed to the community leader, for permission

*Corresponding author: olammauchenna@gmail.com

and mobilization of the people of Iva valley. The study population were properly sensitized on the mode and intent of the study.

3) Study design and sampling

The work was a cross-sectional study of the population (carried out during the month of November) to ensure that every group was represented, in order to determine malaria infection across all age groups.

4) Malaria Prevalence Study

The participants in the study were apparently healthy individuals who did not show any of the common signs of malaria. Their biodata such as names, ages, sex and occupations were collected through oral interview and recorded in a field note book.

5) Blood sample collection

Blood sample was collected using venipuncture technique. 9, 10 Soft tubing tourniquet was fastened to the upper-arm of the patient to enable the index finger feel a suitable vein. The puncture site was then cleansed with methylated spirit (methanol) and venipuncture made with the aid of a 21g needle attached to a 5 ml syringe. When 2ml blood had been collected, the tourniquet was released and the needle removed immediately while the blood was transferred into an EDTA bottle [10].

Collection of blood sample starts with the cleaning of a patient's finger is cleaned with 70% ethyl alcohol, allowed to dry and then the fingertip is pricked with a sharp sterile lancet and two drops of blood are placed on a glass slide. The thick blood film is prepared by placing a blood spot which is stirred in a circular motion with the corner of the slide, taking care not make the preparation too thick, and allowed to dry without fixative. After drying, the spot was stained with diluted Giemsa (1: 20, vol/vol) for 20 min, and washed by placing the film in buffered water for 3 min. The slide is allowed to air-dry in a vertical position and examination using a light micro- scope. As they are unfixed, the red cells lyse when a water-based stain is applied. A thin blood film is prepared by immediately placing the smooth edge of a spreader slide in a drop of blood, adjusting the angle between slide and spreader to 45 and then smearing the blood with a swift and steady sweep along the surface. The film is then allowed to air-dry and is fixed with absolute methanol. After drying, the sample is stained with diluted Giemsa (1: 20, vol/vol) for 20 min and washed by briefly dipping the slide in and out of a jar of buffered water (excessive washing will decolorize the film). The slide is then allowed to air-dry in a vertical position and examined under a light microscope [11].

3. Identification of Malaria Parasites

1) Microscopy

Thick-blood films were prepared according to the technique outlined by Baker12, 13, 10. A drop of each blood sample was placed in the centre of a grease-free clean glass slide. The blood was homogenously spread out in a circular motion using an edge of a spreader slide to make an even smear. The slide was kept for air-drying and staining with field's stain. The slide was held with the dried thick film side facing downward and dipped in field's stain A (eosin) for 5 seconds. It was washed off gently in clean water and then dipped in field's stain B (methyl azure) for 5 seconds and washed again in clean water. The stained films were examined under a microscope using X100 objective for the presence of malaria parasites.

2) Care-Start rapid diagnostic testing

Malaria Rapid Diagnostic Test kit (Care Start Tm Malaria HRP2 One Step Rapid Test) was used in the field to detect malaria infections. Whole blood (5µl) was added into sample wells and 60µl (2 drops) of assay buffer were added into assay buffer wells. The blood-buffer mixture was allowed to run toward the test and control window. Result was read within 20 minutes. The presence of two colour bands (One on the control and one on the test) indicated positive result. The presence of only one band (the control line) within the result window indicated negative result while the presence of only one band on the test line indicate an invalid result.

3) Statistical analysis of data

Data on blood samples and mosquitoes collected were analyzed using Social Sciences Statistical Package (SPSS) version 17.0 and chi square. The sampling error was taken to be 95% Confidence interval.

4. Results

A total of 379 persons examined in Iva valley sub-urban area, 169 (44.6%) were positive for malaria parasite using microscopy while CareStart RDT recorded 43.80% malaria prevalence in the area (Table 1). In the different study location in the in Iva valley, Camp one recorded the highest prevalence of 46 (27.2%) while Forest hill recorded the lowest prevalence rate of 22 (13.0%). The difference between the various residential location was however not significant (X2 calc = 2.8447, X2 tab = 9.837, P>0.05).

table

Table 1
Prevalence of malaria in the study area according to communities

Location	No examined	Positive (Microscopy) (%)	Positive (RDT) (%)
Camp One	100	46 (27.2)	42 (25.3)
Camp two	51	25 (14.8)	22(13.2)
Valley road	100	38 (22.5)	42(25.30)
Forest Hill	44	22 (13)	20(12.04)
Pottery	84	38 (22.5)	40(24.09)
Total	379	169 (44.6)	166 (43.8)

Table 2
Prevalence of Malaria in relation to Gender in the Study areas

Gender	Number Examined	Number Positive by Microscopy (%)	Positive by RDT (%)
Female	248	117(69.2)	108(65.1)
Male	131	52(30.8)	58(51.2)
Total	379	169 (44.6)	166 (43.8)

Malaria prevalence in gender and age groups in the study area using microscopy. Females have higher prevalence of 69.2% than males with prevalence of 30.8% (Table 2). However the difference in gender prevalence was not significant (X2 calc = 1.939, X2 tab=3.841, P>0.05). The study

showed that the age group 11 -15 years had the highest prevalence rate of 27.8%, followed by 16- 20 with 15.98%. The age group 21 - 25 years had the least prevalence rate of 1.18%. The mean age of the sampled population was 16.7+ 21.47 years (Table 3).

Table 3
Prevalence in relation to Age in the Study Areas using microscopy

Age Group	Number Examined	Number Positive (%)
1-5	26	14 (8.3)
6-10	75	26 (15.4)
11-15	103	47 (27.8)
16-20	52	27 (16.0)
21-25	14	2 (1.2)
26-30	12	4 (2.4)
31-35	13	9 (5.3)
36-40	25	8 (4.7)
41-45	10	5 (3.0)
46-50	14	10 (5.9)
51-55	18	5 (3.0)
56-60	9	7 (4.1)
>60	8	5 (3.0)
Total	379	169

Table 4
Evaluation of CareStart RDT using microscopy as gold standard

Test	Malaria +ve	Malaria –ve	Total
RDT +ve	151	15	166 (43.80%)
RDT –ve	18	195	213 (56.20%)
Total	169(44.6%)	210 (55.4%)	379

Table 5
Sensitivity, Specificity and the Predictive Value of Care-start RDT

Test	Malaria +ve	Malaria –ve
RDT +ve	151	15
RDT –ve	18	195
Sensitivity	89.35%	
Specificity	92.86%	
Positive Predictive Value	90.96%	
Negative Predictive Value	91.55%	

Malaria Prevalence using Care-start RDT and microscopy as gold standard. Care Start RDT kit indicated that 43.80% (166/379) of the sampled population were infected with malaria parasite and 44.6% (169) were positive by microscopy (Table 4). Of those positive by microscopy (n=18) were negative by RDT (false negatives), while 9.0% of those positive by RDT (n=15) were negative by microscopy (false positives).

Determination of the Sensitivity, Specificity and the Predictive Value of Care-start RDT.

The sensitivity of the CareStart RDT test kit was 89.35%, the specificity was 92.86%, while the positive and negative predictive values were 90.96% and 91.55%, respectively when compared to microscopic examination of blood films as gold standard for detection of malaria (Table 5). NB: Sensitivity = True positive/ (True positive + False negative) x (100); Specificity = True negative / (True negative + False positive) x (100); Positive predictive Value = True positive/ (True positive + False positive) x (100); Negative predictive value= True negative/ true negative +false negative x 100.

5. Discussion

There was unequal representation of the participants from the communities (131 Males, 248 Females). This could be attributed to health seeking and treatment behavior often displayed by the female group. The higher prevalence recorded among the female can be found in other studies reported in Awka and Abeokuta. 14 However, at 5% level of significance, the difference was not statistically significant (P>0.05). This agrees with other findings who reported that sex did not affect malaria prevalence among individuals [15]. There was also unequal representation in the age group, the highest number of participants were of the age group 11 – 15 years, 103 (27.2%). This may be attributed to the fact that most of the women who participated in the study came with their children.

A high prevalence of malaria 169 (44.6%) for microscopy and 166 (43.8%) for RDT was observed among the study participants. The total prevalence of 44.6% reported in this study is higher than other studies. For instance, prevalence of 17% was reported in Eastern Nigeria 16, 27.3% prevalence in Sokoto.17 Although the prevalent is lower when compared to other studies: 46% prevalence in Nnewi, 18 76% in Azia, Anambra State, 19 72% prevalence in Osogbo, 20and 80% prevalence in Awka.14 From the findings, it shows that malaria is prevalent in Iva valley sub-urban although the population involved in the study were obviously healthy individuals at the time of the study. Malaria infection was recorded from all the five communities with Camp one recording the highest prevalence of 46 (27.2%) while Forest hill recorded the lowest prevalence rate of 22 (13.0%). The difference between the various residential location was however not significant (X2 calc = 2.8447, X2 tab = 9.837, P>0.05). This is an indication of widespread end emicity of malaria in the community.

Furthermore, a higher percentage prevalence of malaria using microscopy was recorded (44.6%) than that of RDT (43.8%). The sensitivity of 89.35% of CareStart RDT testkit was fairly reasonable while specificity of 92.86% was significantly high. The finding in the current study was consistent with the findings in China with a sensitivity of 89.68% 21though slightly higher than the findings from other studies conducted in Northern Nigeria with a sensitivity of 78.4%, 22 82% sensitivity and 91% specificity. 23 The false positives recorded can be attributed to the fact that both children and adult involved in the study may have received some anti-malaria before presentation were not excluded in this study, and HRP-2 is known to persist in the blood for a few weeks after treatment. Thus the test may still remain positive even when the parasites have been cleared. The positive predictive value of 90.96% recorded in this study meant that the kit has the capability of confirming malaria with a precision of 91%, while the negative predictive value of 91.55% means that the RDT is good in ruling out malaria, thus giving the clinician the confidence that a negative test excluded malaria in about 92% of cases. The data collected in this study was not linked to patients' medical records. Therefore, it was impossible to collect clinical information, such as recent intake of antimalarials or presence of other factors, which may have influence on the results. However it is important to note that the data described were obtained in true field conditions, i.e., conditions that were suboptimal in terms of RDT storage and handling and staff expertise. It is known that such factors

influence RDT performance [24, 25]. Several factors such as the manufacturing process, environmental conditions may affect RDT performance.26, 27 Manufacturers usually recommend 4°–30°C as the optimal temperature range. In practice, exposure of RDTs to > 70% humidity and/or > 30°C frequently occurs in the tropics. Although the figures reported in this study was less than the 95% recommended by the World Health Organization (WHO), and though microscopy is the gold standard there is still possibility of human error and technical problem of microscopic identification of parasites which could explain the disparity between the two diagnostic methods.

6. Conclusion

CareStart TM HRP2 Rapid Diagnostic Test (RDT) kit has good sensitivity and specificity when compared with the gold standard Light Microscopy. The use of CareStart HRP-2 RDT in health facilities especially in areas without access to microscopy or where standard laboratories and trained microscopist are not available should be encouraged rather than depending on presumptive treatment. The government likewise can encourage diagnosis by subsidizing the various RDTs that have good sensitivity and specificity. Its deployment for use at the community level and patent medicine stores should be encouraged as people patronize them more than the comprehensive health facilities.

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