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Original Research Article

Rapid diagnostic test versus microscopy in the diagnosis of acute malaria in a district hospital in Enugu state, Southeast Nigeria

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ABSTRACT

Background: Malaria is a systemic disease caused by various species of *Plasmodium*, transmitted through the bite of a female *Anopheles* mosquito. According to the World Health Organisation, there were 214 million cases of malaria worldwide in 2015. Nigeria's burden of malaria is about 51million cases and 207,000 deaths annually, accounting for 60% of outpatient visits to hospitals, 11% of maternal mortality, and 30% of child mortality. The study aimed to compare RDT and microscopy in malaria diagnosis in a District Hospital in Enugu state, Southeast Nigeria.
Methods: Blood samples of 300 suspected cases of acute malaria were tested for malaria parasite using RDT and microscopy simultaneously.
Results: In 2017, the study found a malaria prevalence of 25% (46.2% in children, and 18.1% in adults) in Awgu. RDT was positive in 38% and microscopy in 70.3% of cases. Both RDT and microscopy were positive in 36.3%, negative in 28.3%, and discordant in 35.4%. Sensitivity of RDT was 50.7% (89.4% in children, and 25.6% in adults). RDT had a specificity of 100% (both children and adults), positive predictive value of 1 (both children and adults), and negative predictive value of 0.6 (0.5 in children, 0.6 in adults).
Conclusions: RDT (SD Malaria Ag P. f) had more sensitivity in children (89.4%) than adults (25.6%), and the occurrence of false negative results was more in adults (46.8%) than children (9.5%). All negative RDT results need to be examined microscopically, to rule out false negative cases.

Keywords: Rapid, Test, Microscopy, Diagnosis, Malaria, Hospital

INTRODUCTION

Malaria is a systemic disease caused by various species of *Plasmodium* (*P. falciparum, P. ovale, P. vivax, P. malariae* and *P. knowlesi*) transmitted through the bite of a female *Anopheles* mosquito. The most serious and sometimes fatal type of malaria is caused by *Plasmodium falciparum*.[1] The greatest burden of the disease is borne by the African region. According to the latest estimates by the World Health Organisation (WHO), there were 214 million cases of malaria worldwide in 2015, with the African region accounting for about 88%, the South-East Asia region 10%, and the Eastern Mediterranean region 2%.[2] However, the 2015 WHO report also indicates that between 2000 and 2015, the incidence of malaria has actually fallen by 37% globally, and by 42% in Africa; while mortality over the same period fell by 60% globally and 66% in the African region.

In Nigeria, despite concerted efforts by the Government of Nigeria and other international bodies (the World Bank, UK's DFID, Global Fund, USAID, among others) to curb the menace of malaria, it continues to be a major public health problem, accounting for more cases and deaths than any other country in the world. Nigeria's burden of malaria is about 51 million cases and 207,000

deaths reported annually, accounting for 60% of outpatient visits to hospitals, about 11% of maternal mortality and 30% of child mortality, especially among children less than 5 years.[3,4]

The greatest prevalence of malaria in Nigeria was reported in the Southwest, North Central and North West (about 50% in children aged 6-59 months), while in the Southeast region the prevalence was 27.6%.[5] The economic burden of malaria in the country is estimated to be about 132 billion Naira (~700 million USD) annually.[6,7] In Enugu state, the average household expenditure per case of malaria is about 12.57 USD and 23.20 USD for outpatient visits and inpatient stays respectively.[8]

Clinical diagnosis of uncomplicated malaria is challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other common, as well as potential life-threatening viral, bacterial, and other febrile illnesses.[1] However, the earliest symptoms include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhoea, nausea, vomiting, anorexia and pruritus.[9] Correct and prompt diagnosis of malaria is crucial to its proper management in order to reduce both complications and possible mortality from it.[10,11]

Since 2010, the WHO has recommended that all suspected cases of malaria be confirmed parasitologically by microscopy or RDTs before treatment, irrespective of age and transmission setting.[2] Malaria diagnosis involves identifying malaria parasites or antigens/products in patient's blood.[1] To date, microscopy for malaria parasite using thick and thin blood films remains the gold standard for the diagnosis of acute malaria. According to WHO, in 2010, there were about 165 million microscopic examinations worldwide.[12] Estimates of the diagnostic sensitivity of microscopic slide evaluation vary according to the type of infecting species, geographic area, and other factors, although generally it is not more than 75%.[12] For patients with non-falciparum malaria, low-level parasitaemia, or partial immunity, or those that have been partially treated for malaria, the diagnostic sensitivity is likely to be even lower than 75%. Sometimes when the parasites cannot be found in peripheral blood smear from patients with malaria, the microscopic demonstration of the presence of malaria pigment in leucocyte (which is a pathognomonic sign of malaria infection) particularly in low transmission areas can be relied upon.[13] The advantages of microscopy in the diagnosis of malaria, includes its ability to identify the infecting species and determine the magnitude of parasitaemia, its usefulness for serial examinations to monitor the efficacy of therapy, and its comparative affordability.[16] However, the shortcomings of microscopic diagnosis of malaria include that it is labour-intensive, time-consuming, requires expertise and its relatively low sensitivity at low parasite level, making it unsuitable for high through-put-use and species determination at low parasite density.[14] Because of these obvious shortcomings of traditional microscopic diagnosis of malaria, it became necessary to look for alternative methods of diagnosis that would eliminate or minimize these shortcomings. One of such newer methods of diagnosis of acute malaria, among others, is the antigen-based rapid diagnostic test (RDT) for malaria. RDTs are fast, easy to perform and do not require specific equipment.[15] Unlike the conventional microscopic diagnosis, RDTs are all based on the same principle and detect malaria antigen or enzymes (e.g. *Plasmodium* histidine-rich protein 2 [HRP 2] which is specific to *P. falciparum* and *Plasmodium spp* lactose dehydrogenase [pLDH], which may be species specific or pan-specific) in blood flowing along a membrane containing specific anti-malaria antibodies.[1,16] The advantages of RDT over the traditional microscopy in the diagnosis of malaria consists in its simplicity, ease of use by non-laboratory technicians/technologists, greatly reduced time of diagnosis and its availability in limited resource settings. However, the disadvantages of RDTs include the occasional occurrence of false positive results (especially in persisting HRP 2 antigenaemia, in cross-reactivity with auto antibodies such as rheumatoid factor), its lower sensitivity compared to reference microscopy, its inability to determine infection with other species of plasmodium (*P. ovale*, *P. malariae*, *P. knowlesi*), its inability to quantify the parasites and the probability of a prozone effect occurring with HRP 2-based RDTs.[17-19]

In our setting (a District Hospital), RDT has been the Mainstay of diagnosis of acute malaria since 2010. However, the observed high rate of false negative results associated with use of RDT (SD Malaria Antigen P. f) diagnostic, even when clinical symptoms are suggestive of acute malaria, necessitated this comparative study of RDT and traditional microscopy to assess the incidence of false RDT negative results. This study will be significant in determining the actual malaria disease burden in the locality, and by extension the state, to help the health system plan and budget for interventions on the reduction of the scourge of malaria.

The aim of the study is to compare RDT and microscopy in malaria diagnosis in our District Hospital, and to underscore the importance of complementing RDT with conventional microscopy in malaria diagnosis, especially in situations when RDTs are negative.

METHODS

The study site, District Hospital Awgu, is situated in Awgu Local Government Area in Enugu West Senatorial District, which is located approximately between latitudes 06 00' and 06 19' North of the Equator and longitudes 07 23' and 07 35' East of the Greenwich Meridian. The LGA is made up of 20 towns and has a population of 390 681.[20]

The topography of Awgu and amount of annual rainfall are such that encourage the breeding of mosquitoes, hence the high local incidence of malaria of 25% (1475 [mean] out of 5881 patients seen annually). 1475 malaria cases constitute the study population. The sample size was calculated using the following formula:

$$n=\frac{Z^2 P(1-P)}{d^2}$$

where n= sample size, Z= Z statistic for a level of confidence, P= expected prevalence or proportion (In proportion of one, if 20%, P=0.2) and d=precision (In proportion of one, if 5%, d= 0.05. Z-statistic: for level of confidence of 95% which is conventional, Z value=1.96.[21]

From the formula, a sample size of 288 was obtained, but for convenience, the final sample size used for the study was 300, obtained by using systematic random sampling technique. Each day, five samples of blood of patients with suspected malaria, got by selecting one out of every two cases, were examined by both RDT and microscopy. Samples were collected three times a week until the required sample size.

For microscopy, thick blood films were prepared using an adaptation of the methods of.[22,19] Each patient's finger was cleaned with 70 ethyl alcohol, allowed to dry and then the side of finger pricked with a sharp sterile lancet and two drops of blood placed on a glass slide. The blood spot was stirred in a circular motion with the corner of the slide, allowed to dry at room temperature without fixative for about one hour and exposed to a Coplin jar prior to staining. After drying, the spot was stained with diluted Giemsa (1:20 vol/vol) for 20 minutes and washed by placing the film in buffered water for three minutes. The slide was then allowed to air-dry in a vertical position, and using a light microscope, a minimum of 100 immersion fields (x100-objective) were examined.

For the RDT, SD Malaria Ag P. f kits manufactured by Standard Diagnostics, Republic of Korea, were used. After cleaning the finger tip, the lateral side of the patient's finger was pricked with a sterile lancet. With a capillary pipette (5 µl) whole blood specimen was drawn up to the black line and then transferred into the round specimen well. 4 drops of the assay diluent were dropped vertically into the square assay diluent well. After 15 minutes, the results were read. In case of negative results, another reading was done at 30 minutes. The presence of one coloured band (control line "C") within the result window indicated a negative result. The presence of two coloured bands (test line "P. f" and control line "C") within the result window, regardless of which band appears first, indicated a positive result.

Data were collected from June 2017 to November 2017. The data were analysed as frequency distributions and using the 2x2 approach, categorizing the results as positive or negative in testing for sensitivity, specificity, and predictive values.[23]

RESULTS

Blood samples were collected from each of the 300 patients (95 children and 205 adults) with suspected acute malaria that were recruited into the study. Each patient's blood samples were examined by RDT and microscopically for malaria parasites. Table 1 shows the distribution of the study participants by age. As shown in the table, 95 (31.7%) of the 300 patients were children, while 205 (68.3%) were adults.

Table 1: Distribution of patients by age.

Children (<18 years)	Adults (≥18 years)
95 (31.7%)	205 (68.3%)

Table 2 shows the prevalence of malaria in District Hospital Awgu in 2014, 2015, and 2016. As shown in the table, the prevalence of malaria was 20.7% in 2014, rising to 27.9% in 2015, and falling slightly to 26.3% in 2016. The mean prevalence for the three years was 25%.

Table 2: Prevalence of malaria in the District Hospital.

Year	Number of cases seen	Total number of patients seen	Prevalence (%)
2014	1126	5451	20.7
2015	1567	5618	27.9
2016	1732	6574	26.3
Mean	1475	5881	25

The prevalence of malaria in children is as shown in Table 3. From the table it is evident that the prevalence of malaria rose steadily from 34.8% in 2014 to 46.7% in 2015, and 57.1% in 2016. Mean prevalence for the three years was 46.2%.

Table 3: Prevalence of malaria in children in the District Hospital.

Year	Children (with malaria)	Total number of children seen	Prevalence (%)
2014	438	1260	34.8
2015	604	1293	46.7
2016	745	1304	57.1
Mean	595.7	1285.7	46.2

Table 4 shows the prevalence of malaria in adults in the District Hospital for three years. According to the table, the prevalence of malaria in adults was 16.4% in 2014. This rose to 22.3% in 2015, but fell drastically to 15.7% in 2016. Mean prevalence of malaria for the three years was 18.1%.

Table 4: Prevalence of malaria in adults in the District Hospital.

Year	Adults (with malaria)	Total number of adults seen	Prevalence (%)
2014	688	4191	16.4%
2015	963	4325	22.3%
2016	987	6270	15.7%
Mean	879.3	4928.7	18.1%

The results of RDT tests compared to microscopy are as shown in Table 5. The table shows that RDT was positive in 38% of cases, while in 62% of cases negative. On the other hand, microscopy was positive in 70.3% of cases, and negative in 29.7% of cases.

Table 5: RDT vs. microscopy results.

	RDT (%)	Microscopy (%)
Positive	114 (38)	211 (70.3)
Negative	186 (62)	89 (29.7)

Table 6 shows the degree of agreement and disparity between RDT and microscopy. From the table, it is evident that both RDT and microscopy were positive in 36.3% of cases, negative in 28.3% of cases, and discordant (negative RDT, but positive microscopy) in 35.4% of cases. The results are dispersed (when both are positive, negative and when discordant) approximately in the ratio of 1.3:1:1.3.

Table 7 shows the degree of agreement and disparity between RDT and microscopy in children and adults segregated. As shown in the table, both RDT and microscopy were positive in 80% of cases in children, negative in 10.5%, and discordant (negative RDT and positive microscopy) in 9.5%, representing a ratio of 8.4:1.1:1. The table also shows that both RDT and microscopy were positive in 16.1% of cases in adults, negative in 37.1%, and discordant in 46.8%, in the ratio of 1:2.3:2.9. The table further shows that the rate of detection of cases of true positives was 5 times higher in children than in adults, while the rate of detection of false negatives was 5 times higher in adults than children.

Table 6: Agreement/disparity between RDT and microscopy.

Positive (RDT/ Microscopy)	Negative (RDT/ Microscopy)	Discordant (-RDT, +Microscopy)
109 (36.3%)	85 (28.3%)	106 (35.4%)

Table 7: Agreement/disparity between RDT and microscopy in children and adults.

	+(RDT/Microscopy)	-(RDT/Microscopy)	Discordant (-RDT,+Microscopy)
Children	76 (80%)	10 (10.5%)	9 (9.5%)
Adults	33 (16.1%)	76 (37.1%)	96 (46.8%)
	80/16.1=5		46.8/9.5=5

Table 8: True disease status (malaria) using microscopy as standard.

Test result	Disease present (+)	Disease absent (-)	Total
Positive (+)	109	0	109
Negative (-)	106	85	191
Total	215	85	300
	Sensitivity 50.7%	Specificity 100%	
	PPV (+) 1	NPV (-) 0.6	

Key: PPV= positive predictive value, NPV= negative predictive value.

Table 9: True disease status in children using microscopy as standard.

Test result	Disease present (+)	Disease absent (-)	Total
Positive (+)	76	0	76
Negative (-)	9	10	19
Total	85	10	95
	Sensitivity 89.4%	Specificity 100%	
	PPV (+) 1	NPV (-) 0.5	

Key: PPV= positive predictive value, NPV= negative predictive value.

Table 8 describes the participants status (disease present, or absent) in relation to the test results (positive, or negative). From the table it is seen that in 109 of cases in which the disease was present, the test result was positive (true positive), in no case where the disease was absent was the test result positive (false positive). In 106 cases

in which the diseases was present, the test result was negative (false negative), and in 85 cases in which the disease was absent, the test result was negative (true negative). Generally, the sensitivity of RDT using microscopy as the standard was 50.7%, specificity 100%, positive predictive value1, and negative predictive value 0.6.

Table 9 shows the disease status in relation to the test results in children. As shown in the table, 76 cases in which the disease was present, had positive test results (true positive), no cases of absence of diseases had positive result (false positive), 9 cases in which the disease was present had negative test results (false negative), and in 10 cases in which the disease was absent, the test results were negative (true negative). Sensitivity of RDT in children was 89.4%, specificity 100%, positive predictive value 1, and negative predictive value 0.5.

Table 10: True disease status in adults using microscopy as standard.

Test result	Disease present (+)	Disease absent (-)	Total
Positive (+)	33	0	33
Negative (-)	96	76	172
Total	129	76	205
	Sensitivity 25.6%	Specificity 100%	
	PPV (+) 1	NPV (-) 0.6	

Key: PPV= positive predictive value, NPV= negative predictive value.

Table 10 shows the disease status in relation to the test results in adults. As shown in the table, in 33 cases in which the disease was present, the test results were positive (true positive), no cases of disease absence showed positive test result (false positive), 96 cases in which the disease was present showed negative test results (false negative), 76 cases in which the disease was absent showed negative test results (true negative). Sensitivity of RDT in adults was 25.6%, specificity 100%, positive predictive value 1, and negative predictive value 0.6.

DISCUSSION

The MDG target for malaria was 75%, i.e. reduction of the scourge of malaria (cases and deaths) between 2005 and 2015 by 75%.[24] The mean prevalence of malaria in Awgu for the 2014-2016 period as revealed by the study was 25% (46.2% for children and 18.1% for adults). At 25%, the prevalence of malaria as found by this study is much higher than the reported 6.8% for the general population (all age groups) in the rainy season.[25] This abnormally high prevalence in the area of study could be accounted for by the type of topography, excessively high amount of annual rainfall, inadequacy of malaria prevention and control measures, among others.

The choice of the most appropriate test for malaria diagnosis may be determined by the level of malaria endemicity (including species), the urgency of diagnosis, and availability of personnel and financial resources.[11] Since 2010, the Mainstay of malaria diagnosis at District Hospital Awgu has been RDT. The present study did a comparison of RDT and traditional microscopy in 300 suspected cases of malaria seen between June and November 2017. Generally, RDT was positive in 114 cases out of 300 (38%), while in 211 of 300 (70.3%) cases, microscopy was positive. These findings compare to what had been reported by.[26] The study further revealed that in 36.3% of cases both RDT and microscopy were positive, in 28.3% both were negative, and in 35.4% both were discordant (negative RDT and positive microscopy).

The degree of agreement between RDT and microscopy is not the same for children and adults. In children, RDT and microscopy were both positive in 80% of cases (true positives [TP]), negative in 10.5% (true negatives [TN]), and discordant (negative RDT and positive microscopy) in 9.5% of cases (false negatives [FN]). On the other hand, in adults, both RDT and microscopy were positive in 16.1% (TP), negative in 37.1% (TN), and discordant (negative RDT and positive microscopy) in 46.8% (FN). This clearly demonstrates that the rate of detection of TPs in children was 5 times higher than in adults, whereas the occurrence of FNs was 5 times higher in adults than in children. The reason for this is not quite understood. However, it could be speculated that in adults, there could be the presence of antibodies similar in nature to RDT HRP 2 that react with the *Plasmodium* antigens in the blood sample. Depletion of *Plasmodium* antigen concentration in the blood may therefore lead to FN results in RDT diagnosis of malaria. These suspected antibodies could be absent in children, hence the occurrence of FN RDT results more in adults than in children. It could also be that these adults are infected more by species of *Plasmodium* (*P. ovale*, *P. malariae*, *P. knowlesi*) other than *P. falciparum*.

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of RDT for children also differ from those of the adult patients, as was the case with the degree of agreement between RDT and microscopy in children and adults. In children, the sensitivity of RDT found by this study was 89.4%, specificity 100%, PPV of 1 (100%) and NPV of 0.5

(50%). In adults, the study found RDT sensitivity of 25.6%, specificity of 100%, PPV of 1 (100%) and NPV of 0.6 (60%).

Sensitivity of a test is defined as the ability of that test to detect the proportion or percentage of people with the diseases who will have a positive result.[27,28] It is calculated with the formula, TP/(TP + FN).

Specificity of a test is the ability of the test to detect true negative cases, i.e. the proportion of people without the disease who will have a negative result, calculated as TN/(TN+ false FP).

PPV of a test is the proportion of the test results that are truly positive i.e. proportion of people with a positive test result who actually have the disease, calculated as TP/(TP+FP).

NPV is proportion of the test results that are truly negative, i.e. the proportion of people with a negative test result who do not have the disease, calculated as TN/(TN+FN).

In the present study, generally, RDT compared to microscopy was found to have a sensitivity of 50.7%. Comparative findings had been reported by some researchers around the country.[29,30] Others have reported extremely lower sensitivity of RDT.[31,32,26] Some of the observed low sensitivity results may have been caused by product instability at temperatures higher than 30^0 C, or by quality control issues during manufacturing.[33]

The study found a specificity of 100% for RDT, comparable with findings from other past studies.[29,31,32] The study further demonstrated a PPV of 1 (100%) and an NPV of 0.6 (60%). The PPV as found by this study is comparable to what had been reported by.[29,31] However, much lower values were reported by.[30,32] Comparatively low NPVs were reported by.[29-31]

CONCLUSION

Prevalence of malaria in Awgu in 2017 is 25% (46.2% in children, and 18.1% in adults). RDT was positive in 38% of cases and microscopy in 70.3%. Both RDT and microscopy were positive in 36.3%, negative in 28.3%, and discordant in 35.4%. Detection of TPs was 5 times higher in children than adults, but the occurrence of FNs was 5 times higher in adults than children. Sensitivity of RDT was 50.7% (89.4% in children, and 25.6% in adults). Both in children and in adults, specificity was 100%. RDT had a PPV of 1 (in both children and adults), and negative an NPV of 0.6 (0.5 in children, and 0.6 in adults).

The findings from the study suggest that RDT (SD Malaria Ag P. f) had more sensitivity in children (89.4%) than adults, and that the occurrence of false negative results was more in adults (46.8%), than in children.

To rule out FN RDT results, it is recommended, therefore that all negative RDT results, especially in adults, where the incidence of false negative results was higher, be examined microscopically.

Limitations of the study

The study participants were not equally distributed by age (there were more adults than children), and this might have affected the results of the study (sensitivity, specificity, and predictive values of RDT).

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