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The performance of an Histidine rich protein-2 rapid diagnostic test (RDT) against the standard microscopy in the diagnosis of malaria parasitaemia among febrile under-five children at Nnewi

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Abstract: *Background:* Malaria remains a major cause of morbidity and mortality, thus there is need for quick, reliable inexpensive diagnostic tool to facilitate its prompt treatment especially in resource poor settings.
Objectives: To compare the sensitivity of a locally available Histidinerich protein-2 based rapid diagnostic test (RDT) with the standard microscopy.
Methods: This study was carried out to test the performance of an histidine rich protein -2 rapid diagnostic test (RDT) against the standard microscopy in the diagnosis of malaria among febrile under-five children attending Paediatric Clinic of NAUTH Nnewi. A total of 200 children under the age of five years were recruited for the study. Data on socio-demographic characteristics and symptoms were collected through an interviewer administered questionnaire. Blood sample was collected in EDTA bottle after observing universal precautions. All of them were tested with both Giemsa stained blood smear and Histidine rich protein-2 (HRP-2) rapid diagnostic test (RDT).
Results: There were 118 males and 82 females, giving a male: female ratio of 1.44:1. Their ages ranged from 3-59 months and the average age was 27±17.49 months. Average number of days the subjects had fever before presentation were 3.78±1.95 days with a range of 1-14 days. Body temperature ranged from 35.9-40.4^{0}C with average of 37.7±0.8^{0}C. Forty (20%) were positive by microscopy while 42 (21%) were positive by rapid diagnostic test. Twenty-percent of those positive by microscopy (n=8) were negative by RDT while 23.8% of those positive by RDT (n=10) were negative by microscopy. Using microscopy as a gold standard, the sensitivity of the RDT was 80%, the specificity was 93.8%. The positive and negative predictive values were 76.2% and 94.9% respectively.
Conclusion: Based on these findings, the RDT demonstrated reasonable concordance with microscopy and was recommended for use at every level of healthcare in the diagnosis of malaria.

Key words: Malaria, RDT, Microscopy, under- five.

Introduction

Malaria is an acute infectious disease caused by protozoa, Plasmodium and transmitted by the bite of an infected female Anopheles mosquito, the vector. It is an important parasitic disease in humans, especially in the tropics and subtropics, with the potential of causing very severe or fatal illness in children and adults. Most worrisome are the consequences amongst children under the age of five years, in whom it constitutes a major cause of morbidity and mortality[1,2]. Pregnant women and non-immune subjects are also significantly vulnerable to the severe and potentially fatal forms of the disease[1,2]. In Nigeria the prevalence of malaria is high, and it is one of the leading causes of morbidity and mortality in the country, accounting for 25% of infant mortality and 30% of childhood mortality[2-4]. Yet, the vast majority of infections are not identified due to problems bordering

mainly on laboratory diagnosis, and except the diagnosis of malaria is given the desired priority, the battle for control of malaria in the developing countries may remain insurmountable.

The Roll Back Malaria initiative emphasizes prompt and accurate diagnosis of malaria as the key to effective management of this childhood scourge[5]. Clinical diagnosis of malaria is imprecise, but remains the basis for therapeutic care for the vast majority of febrile patients in malaria endemic areas, where most of the time, laboratory diagnosis is out of reach. Despite this obvious need for improvement, malaria diagnosis is the most neglected aspect of malaria research accounting for less than 0.25% of the 323 million dollars invested in malaria research, in 2004[6]. Yet, rational therapy of malaria requires a diagnostic tool with "decent" sensitivity and specificity values, thus preventing wastage of treatment cost.

Confirmatory diagnosis before initiation of treatment in recent times regained attention, a trend that was somewhat influenced by the spread of drug resistance and thus the requirement of more expensive drugs[7]. Microscopic examination of Giemsa stained blood smear has always been the gold standard of malaria diagnosis. It is a very suitable instrument for malaria diagnosis and control, because it is inexpensive to perform, capable of differentiating between the malaria species and indeed, in quantifying parasite density. However, it is encumbered by the fact that it requires a well trained and competent microscopist, rigorous maintenance of functional infrastructure plus effective quality control and quality assurance, in addition to regular power supply. The import of the potential limitation posed by poor expertise is clearly worse in the developing countries but even in developed countries, expert microscopists are scarce.

A malaria rapid diagnostic test (MRDT) is a device that promptly detects malaria antigen in a small amount of blood (about one drop) by immunochromatographic assay with monoclonal antibodies directed against the parasite antigen and impregnated on a test strip.[8]The result is usually a coloured test line and this is obtainable in 5-20 minutes. It does not need sophisticated training, manpower or electricity. It can therefore be used in both urban and rural areas especially where expert microscopist is scarce. Plasmodium falciparum is the predominant cause of malaria in West African subregion[9]. It is the only specie of Plasmodium the produces Histidine rich protein-2, the enzyme detected by most available MRDT.
This study is aimed at comparing the sensitivity of locally available and approved Histidine rich protein-2 based MRDT with microscopy (gold standard), to ascertain and validate its usefulness in the diagnosis of malaria in our environment where expert microscopist is scarce.

Materials and method

The study was carried out at the children's out-patient clinic and Children's Emergency room of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nigeria. The hospital provides a wide range of medical, surgical, diagnostic, out-patient, in-patient, rehabilitative and support services. The hospital maintains a Paediatric out-patients' clinic and a childrens' emergency room services every day.

Nnewi is a commercial city located in Nnewi North Local Government Area of Anambra state, South-East, Nigeria. Its population based on 2006 census estimate is 391,227. The people are predominantly Igbo-speaking and mainly traders and civil servants. Nnewi is located on latitude 6^0 01' N and longitude 6^0 55'E, has a mean daily temperature of 30.4^{o}C, and mean annual rainfall of about 200 cm.[10] It has two main seasonal variations; the rainy season which spans between April and November and the dry season which lasts from November to March each year. Nnamdi Azikiwe University Teaching Hospital is a tertiary institution in the State and enjoys huge patronage and referrals of patients from all over Anambra State and neighbouring states.
The study is a Cross–sectional, hospital based observational study done during the months of May and June 2012. Malaria antigen detected with HRP-2 based RDT (SD bioline) was compared with microscopy results. This study involved children aged 0-59 months attending the children's out-patient clinic and children's emergency room of the Nnamdi Azikiwe University Teaching Hospital Nnewi, presenting with fever defined as axillary temperature of $\geq$ 37.5°C (measured with mercury in glass thermometer), or history of fever within the last 72 hours.

Institutional ethical approval was obtained from the Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital Nnewi. All the subjects for this study who met the inclusion criteria were recruited as they presented at the Children's Outpatients' Clinic or the Children's Emergency Room of the hospital. They were recruited consecutively until the sample size was met.

The data on socio-demographic information as well as symptoms were collected via a questionnaire which was administered to the mother or the caregiver. About 2mls of blood was collected from each participant and put in an ethylene diamine tetracetic acid (EDTA) bottle; aseptic and universal safety precautions were maintained all through. The RDTs were performed by the researcher according to manufacturer's instruction. The thick and thin blood smear were prepared using 3% Giemsa stain according to standard WHO recommendations[11]. The slides were prepared and read by a laboratory scientist who is certified by the World Health Organization The RDTs and microscopy were performed independent of each other and were brought together at the collation stage. The RDT kits were supplied by the manufacturer, SD Bioline, (Standard Diagnostic Incorporated, Altenhostrasse, 80D-66386 St Ingbert, Germany) through

their marketer in Nigeria (Codis pharma limited, 15 Fatai Irawo Street, off Airport Road, Ajao Estate, Lagos, Nigeria Batch No 082112). Quality was assured by keeping the test kits within the temperature range of 25^0C-28^0C which is within the limit recommended by the manufacturers throughout the period of the study.

Data was analyzed using computer software SPSS (version-16 Chicago, IL). The relevant means, standard deviations and percentages were calculated.

Relative usefulness of the RDTs was determined using sensitivity, specificity, positive predictive and negative predictive values.

The well known malaria parasite (MP) detection using standard microscopic technique was used as the gold standard.

Results

A total of 200 children under the age of five years were recruited for the study. There were 118 males and 82 females, giving a male: female ratio of 1.44:1. Their ages ranged from 3-59 months and the mean age was 27±17.49 months. Those in the age range of 12-23 months and 24-35months constituted the highest number (23%) each (Table 1).

Table 1: Age distribution of the subjects

Age (Months)	Total n(%)
0-11	45 (22.5
12-23	46 (23.0)
24-35	46 (23.0)
36-47	19 (9.5)
48-59	44 (22.0)
Total	200 (100.0)

The mean number of days the subjects had fever before presentation were 3.7±1.9 days with a range of 1-14 days. Body temperature ranged from 35.9-40.4^{0}C with mean of 37.7±0.8^{0}C. Fourty (20%) were positive by microscopy, while 42 (21%) were positive by rapid diagnostic test (figure1). Twenty percent of those positive by microscopy (n=8) were negative by RDT, while 23.8% of those positive by RDT (n=10) were negative by microscopy. Table 2 Shows the evaluation of accuracy of RDT in the diagnosis of malaria using microscopy as a gold standard.

Fig 1: Prevalence of malaria by microscopy and RDT

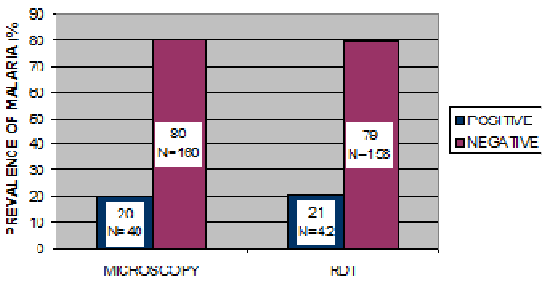

Table 2: Evaluation of accuracy of RDT in the diagnosis of malaria using microscopy as gold standard.

Test	Presence Malaria	Absence of Malaria	Total
Positive	32	10	42
Negative	8	150	158
Total	40	160	200

- Sensitivity =80.0%
- Positive predictive value =76.2%
- False positive rate = 6.2%
- Specificity = 93.8%
- Negative predictive value =94.9%
- False negative rate =20.0%

Note; 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

Using microscopy as a gold standard, the sensitivity of the RDT was 80%, the specificity was 93.8%, while the positive and negative predictive values were 76.2% and 94.9%respectively. False positive was 6.2% while false negative was 20%.The 20% of the subjects which constitute the false positives had a mean parasite density of 731/µL, while the true positive subjects had a mean parasite density of 23,179/µL.

Parasite density ranged from 40-136000/µL with a mean of 18,689/µL. Those in the age range of 0-11 months had the highest parasite density with a mean of 38,144/µL, whereas those in the age range of 12-23 months had the least parasite density with a mean 721/µL . At a density of ≥3350 parasites/µL, sensitivity was 100%, at ≥2000 parasites /µL sensitivity of the RDT was 89.4%, and 62% at ≤500 parasites/µL. However at a density of 100 parasites/µL and below sensitivity was 0%. (Table 3).

Table 3: The frequency distribution of malaria status and sensitivity of the RDT test for malaria at different parasite density cut-offs

Parasite Density and RDT Test Result	Presence of Malaria N	Sensitivity %
Parasite Density < 100		
Positive	0	0
Negative	3	
Parasite Density ≤ 500		
Positive	10	62.5
Negative	6	
Parasite Density ≥2000		
Positive	17	89.4
Negative	2	
Parasite Density ≥ 3352		
Positive	14	
Negative	0	100

Discussion

This study established a high level of concordance between the rapid diagnostic test kit (SD Bioline) used in this study and microscopy. At 80%, the sensitivity

was fairly reasonable while the specificity of 93.8% was significantly high. The implication is that at a sensitivity of 80% the kit is capable of detecting 80 out of 100 children with malaria, i.e. it will miss 20% of the population. Specificity is the ability of the kit to detect false positives implying that the kit has the ability to detect 93 out of every 100 children who do not have malaria. Although these figures are 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 this disparity. Even among expert microscopists, ability vary[12,13]. Another possibility is the fact that those children who 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 76.2% means that the kit has the capability of confirming malaria with a precision of 76%, while the negative predictive value of 94.9% means that the RDT is good in ruling out malaria, thus giving the clinician the confidence that a negative test excluded malaria in about 95% of cases. Although the false positive rate of 6.2% is low enough to be accepted, the false negative rate of 20% is on the high side. Several factors such as low parasite density[14], could be an explanation for this. But in the present study, the subjects that constitute this 20% false negative have a mean parasite density of 731parasites/microlitre which is quite low compared to the mean density of 23,719 parasites/ microlitre of the true positive subjects. Other factors such as deletion or mutation of HRP-2 gene[15] have been reported in some cases as reasons. It has also been suggested that anti-HRP-2 antibodies in humans may explain some false negative tests[16]. The sensitivity and specificity findings in this study are similar to the findings (82% and 91%) by Adesanmi *et al*[17] in a similar study using HRP-2 based RDT among febrile children in Enugu South-East Nigeria. The positive and negative predictive values documented in this study were also similar to Adesanmi's[17] findings. Sani *et al*[18] using an HRP-2 based RDT in Sokoto documented a sensitivity of 90.2% which is higher than the 80% in the present study. It is possible that the older age group (0-12years) involved in Sani's[18] study may have contributed to this disparity because in endemic areas older children with malaria are likely not to be symptomatic until much higher parasite densities are reached than younger children who are less immune to malaria. They however, documented a specificity of 95.4% which is similar to the 93.8% documented in the present study. Ly and co-workers[19] in Senegal documented sensitivity and specificity of 100% and 98.3% for an HRP-2 based RDT. They also documented positive and negative predictive values of 80% and 100% respectively which are little higher than those of the present study, The sensitivity, specificity and predictive values documented in this study is also similar to a study done in India[20] and other studies elsewhere[21,22],

The sensitivity of RDT is usually affected by parasite density and this study is not an exception. The sensitivity of the RDT in the study was noted to be reduced by low parasite density; it was 100% at a density of $\geq$3335 parasites/microlitre, 89.4% at $\geq$2000 and 62% at $\leq$500 parasites/microlitre. (Table 3) This is similar to the findings in other studies.[18,23]Sani *et al*[18] similarly documented that at a density of >1600 parasites/microlitre sensitivity was 100% while it was 62% at a density of <800 parasites/microlitre. In the evaluation of an HRP-2 prototype assay in Thailand and Peru for *P. falciparum*, sensitivity was found to be 100% for parasite density $\geq$500/µL and 83% for <500/µL[24]. The sensitivity of this kit was however noted to drop to 0% at parasite density of 100/microlitre and below. Although this agrees with the recommendation by WHO, the implication is that the kit is not able to detect malaria parasites at such a low density. But even at such low density, children particularly infants may still manifest with symptoms of malaria[25] and relating to this study means that SD bioline will fail to detect *Plasmodium falciparum* at density $\leq$100/µL. This might result in missing some cases of malaria in children especially infants, which could be fatal. However it must be noted that it is only three subjects, representing 7.5 percent of the positive subjects that have parasite density of $\leq$ 100/µL.

Conclusion

Malaria rapid diagnostic test in this study demonstrated a reasonable concordance with microscopy, having achieved a sensitivity of 80% and specificity of 93.8%. It is therefore recommended for use at every level of healthcare delivery, but result must be interpreted with caution since it may not detect malaria in 20% of infected persons.

Authors contribution
ECE and EJC conceived and designed the study. ECE literature review, data collection and analysis, ECE, EJC,UEF, CJO- discussion, AGI and OIO – laboratory analysis.
Conflict of interest: None
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