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Comparative study on the performance of microscopic and rapid diagnostic test (RDT) for malaria among some patients in Maiduguri metropolis

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Abstract

Malaria remains a major public health problem in Nigeria, that a positive, accurate and reliable microscopy or RDT should preferably be obtained before commencing treatment. High specificity will reduce unnecessary treatment with antimalarial drugs and improve the diagnosis. This study analyze the performance of microscopy and Rapid Diagnostic Techniques (RDT) used in the diagnosis of malaria at the University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria from September to October, 2019. A total of 118 blood samples was screened for malaria by preparation of thick and thin film, Leishman staining and microscopic examination of the slides under oil immersion objectives. Rapid Diagnostic test (RDT) was performed using NADAL® Malaria 4 species test cassettes, with emphasis on falciparum malaria. The results shows that 65 patients (55.1%) were positive for malaria, out of which 42 (64.6%) were males, mainly (24.6%) within the young age group (1-10 years). Some of the patients 17 (26.2%) have started taking antimalarial drugs before coming to the hospital. Out of the 96 patients presented with acute malaria, only 43 (66.2%) were positive, but all those with severe cases (33.8%) were positive by both microscopic and RDT tests. In general, 53 patients (81.5%) were positive by microscopy, while only 12(18.5%) are RDT-positive. The study revealed a low performance of RDT with high number of false negative tests compared to microscopy on the same samples. It is therefore, necessary to reinforce training in microscopy, improved supplies and ensure proper handling/storage of the rapid test kits in malaria-endemic area, like Nigeria.

Keywords: Malaria; Microscopy; Rapid Diagnostic Techniques (RDT)

1. Introduction

Malaria is a parasitic disease that accounts for 15% of deaths among children under five years of age [1]. It is prevalent in tropical and sub-tropical region because of rainfall, warm temperatures and stagnant water which provide habitats ideal for mosquito larvae. The World Health Organization (WHO) in 2010 confirm that there were about 219 million documented cases of malaria that kills about 660,000 `yearly and 1.2million people many of which were children in Africa. In the Nigeria, 95.2% of malaria infections were *Plasmodium falciparum*, 9.5% *Plasmodium vivax*, and eight cases mixed infection [2].

There are four (4) species of *Plasmodium* that can infect and be transmitted by humans which includes *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale and plasmodium malariae. Plasmodium falciparum* is one of the species that causes malaria in humans, transmitted by female anopheles mosquito [3]. Malaria cause by this specie (also called

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malignant or *falciparum malariae*) is the most dangerous form of malaria with the highest rates of complication and mortality [4].

As of 2006, there were an estimated 247 million human malaria infections in which 98% in Africa, 70% being among children five (5) years and below. In most African countries almost every malarial death is causes by *plasmodium falciparum* [5]. It is characterized by severe headache which usually progress to general fever. *Plasmodium vivax* is another malarial parasite, frequently and widely distributed, causing recurring benign tertian malaria, that commonly infect humans [6]. It is less virulent than *plasmodium falciparum,* can lead to severe disease and if not treated can lead to death [7]. *Plasmodium vivax* is found mainly in the United States of America, Latin America and Africa which cause massive death (due to pathologically enlarged spleen), but more often it causes debilitating but non-fatal symptoms [4].

Plasmodium ovale and *Plasmodium malariae* cause a general milder form of malaria that is rarely fatal [6]. *Plasmodium ovale* is a species of parasite protozoan that causes tertian malaria in humans it is closely related to *plasmodium vivax* which are responsible for most malaria, but it is rare and substantially less dangerous than *plasmodium falciparum*. *Plasmodium malariae* causes fever that reoccur at approximately three-days intervals (quartan fever), longer than the two days (tertian) hence its alternate names [8].

The classic symptoms of malaria is paroxysm a cyclical occurrence of sudden coldness followed by rigor and then fever and sweaty occurring every two days (tertian fever) in *plasmodium vivax* and *plasmodium ovale* infections and every three-days (Quartan Fever) for *plasmodium malariae. Plasmodium falciparum* infection can cause recurrent fever every 36-48 hours or a less pronounced and almost continuous fever, therefore severe malaria is usually caused by *plasmodium falciparum* with symptoms rises 9-30 days after infection [9].

It is necessary to observe many fields to detect infection, which implies at least two expert microscopists can work on the same sample. However, the quality of microscopy-based diagnosis is frequently inadequate [10]. In many malaria-endemic regions, microscopic diagnosis has certain limitations, including a shortage of skilled microscopists, inadequate quality control, and the possibility of misdiagnosis due to low parasitaemia or mixed infections [11, 12]. In Nigeria, this diagnostic method is unavailable in some rural health facilities, and predictive diagnosis is still widely used

Moreover, sometimes it is difficult to determine the species of *Plasmodium* by microscopy [13]; consequently, some species are not reported in the country, such as *Plasmodium ovale*, which has a morphology similar to *P. vivax*. Even if this does not affect treatment, because the patient will receive the same treatment for both species, it has important implications in malaria epidemiology and mapping [1].

The main strategy for malaria control is quick and accurate diagnosis followed by effective treatment. Early and accurate diagnosis of malaria is essential for both effective disease management and malaria surveillance. The quality of malaria diagnosis is important in all settings, as misdiagnosis can result in significant morbidity and mortality. Microscopy and RDTs are the primary choices for diagnosing malaria in the field [14]. However, false-negative results may delay treatment and increase the number of persons capable of infecting mosquitoes in the community. The present study was aimed to analyze the performance of microscopy and RDTs as the two main techniques used in the detection of malaria infection among patients attending University of Maiduguri teaching hospital.

2. Material and methods

2.1. Study Design

This is a cross-sectional study carried out from September to October, 2019 in University of Maiduguri Teaching Hospital Maiduguri to provide baseline data on malaria prevalence, by screening patients using RDT and microscopic diagnostic techniques.

2.2. Study Area

Maiduguri is the capital city of Borno State in north-eastern Nigeria. It is one of the sixteen LGAs that constitute the Borno Emirate, a traditional state located in Borno State, Nigeria. The highest record temperature was 47 °C (117 °F) on 28 May 1983, while the lowest record temperature was 5 °C (41 °F) on 26 December 1979. Tree planting was a priority of the city's colonial administration, and large trees along major roads give protection from intense sun. Maiduguri is estimated to have a population of 1,907,600, as of 2007. It grew in size by 15.1 square kilometers in 2002-2012. It is located on latitude: 11°51'N; longitude: 013°05'E; and elevation: 354m, 1161'. Languages spoken in the area

include the predominant Kanuri, followed by Fulfulde, Gamargu, Hausa and Shuwa Arab. The University of Maiduguri was founded in 1975.

2.3. Sample collection and processing

A total of 118 blood samples was collected from the finger tip for the diagnosis of malaria using malaria RDTs and microscopy. The samples was processed according to standard laboratory techniques described by [15].

2.3.1. Blood film preparation and staining

Thick and thin film was made directly from the finger-prick blood sample dropped on clean glass slide and allowed to air-dry for 60 seconds. Leishman staining techniques [15] was used to stain the slide.

2.3.2. Microscopic examination

A drop of oil immersion was placed on a stained slide and mounted on a light microscope of low intensity of light, with objective lens of x100 with the aid of drop of oil immersion and was clearly examined for typical diagnostic properties of malaria parasite [11].

2.3.3. Rapid diagnostic test

The NADAL® Malaria 4 species test (Test cassette) (Nal von Minden, Moers, Germany) was used as the RDT in situ. A 5 µl of whole blood was dropped into the sample well. Four drops of the assay diluent were added to the well according to the manufacturer's protocol [16].

2.3.4. Ethical clearance/approval

The study was approved by the University of Maiduguri Teaching Hospital Maiduguri joint ethics. All participants signed an informed consent form that covered sample analysis for Malaria infection. Each participant was given a number and their demographic details recorded. All patients presented with clinical diagnosis of malaria were included in this study.

3. Results

A total of 118 patients were enrolled in this study, 65 (55.1%) were positive for malaria out of which 42 (64.6%) were males, mainly (24.6%) within the young age group (1-10 years). Some of the positive patients 17 (26.2%) had pre-antimalarial treatment before coming to the hospital. Out of the 96 patients presented with acute malaria, only 43 (66.2%) were positive, while all those with severe cases (33.8%) were positive based on both microscopic and RDT tests.

Malaria distribution based on the two diagnostic tests used in this study (table 2), shows that out of the 65 positive cases 53(81.5%) are positive by microscopy, while only 12(18.5%) are RDT-positive.

Table 1 Malaria distribution in the study population according to patients' characteristics

Patients characteristics	**No. of Samples collected (n=118)**	**No. of positive Cases (n=65)**	**Percentage positive (%)**
Age group (years)			
1 – 10	34	16	24.6
11 – 20	23	09	13.8
21 – 30	19	11	16.9
31 – 40	12	11	16.9
41 – 50	15	05	7.7
51 – 60	07	08	12.3
61 – 70	06	03	4.6
Above 70	02	02	3.1

Gender			
Male	81	42	64.6
Female	37	23	35.4
Pre-antimalarial treatment			
Yes	31	17	26.2
No	87	48	73.8
Clinical findings			
Acute/mild case	96	43	66.2
Severe case	22	22	33.8

Table 2 Distribution of Malaria in the study population based on Diagnostic test method

Diagnostic method	No. of positive Cases (n=65)	Percentage positive (%)
Microscopy	53	81.5
Rapid Diagnostic test (RDT)	12	18.5

4. Discussion

Malaria is an important disease that causes mortality and morbidity among both children and adults in endemic countries. It is a leading problems that cause of debilitating illness with over 300-500 million cases and account for about one million death each year worldwide [1]. Prompt and accurate diagnosis of Malaria is part of effective disease management. All patients with suspected cases should be treated based on confirmed diagnosis by microscopy or RDT [1]. Correct diagnosis in malaria-endemic area is particularly important for the most vulnerable population groups, such as young children and immunocompromised individuals in whom malaria can be rapidly fatal [7].

In this study, malaria was commonly found in male 64.6%, mainly children, which can be associated with their vulnerability and exposure to outdoor activities than their female counterpart. Malaria still remains a common cause of morbidity and mortality, especially in children under the age of 5 years [17, 18] in whom immunity to malaria has not yet developed, hence the need for proper case definition and management.

The present study did not observe an increase in positivity rates with age of the patients for both RDT and microscopy as found in a study by [19] and [20]. This is not consistent with findings of [21] where they observed an increase in sensitivity with advancing age. These variations may be due to differences in sample size, socio-economic and the demographic characteristics of the studied populations.

There is an evidence of drug resistance and treatment failure among some of our symptomatic patients (26.2%) tested positive. This due to self-medication and over-the-counter prescription of antimalarial drugs before clinical presentation. The prevalence of acute malaria among patients presumptively diagnosed to have positive cases is higher (66.2%) in our study, which signifies the importance of early diagnosis, prior to therapeutic intervention.

This study found more positive malaria cases for microscopy than RDT, with a low sensitivity but higher specificity rate. Our microscopy positivity rate was similar to the findings of previous studies by [18, 22 and 23], but lower than the report by [24]. However, our RDT positive results was still higher (18.5%) than what was obtained (2.3%) by [21] in the same study area. This was attributed to seasonal variations within the years of study. In Uganda, [25] reported a 78% positivity for microscopy and only 14% for RDT, which can be due to differences in geographical location, sample size, methodology and types of RDT kits used [15].

Some previous studies that have been carried out to compare microscopy and RDT showed both to be sensitive in the diagnosis of malaria [18, 24]. In Uganda, [19] found RDT to be not only sensitive but also more feasible than microscopy.

Our study found a low sensitivity and specificity as against the manufacturer's specifications. This is not in agreement with the findings in Uganda by [26] where RDT was found to have higher sensitivity and specificity. However, [21] found RDT not to be a reliable test in under five children with acute uncomplicated malaria as their sensitivity of 8.3% was also low which is comparable to our findings. Higher sensitivity of 69.6% and 82.0% to RDT was reported by [27] in Enugu and [28] in Lagos, respectively. Variations in sensitivity between the different studies may be due to differences in the types of RDTs used or test methodology and skill of the microscopists.

Another reasons for the low RDT positivity rates may be due to external factors that might have affected the stability of the test such as exposure to extreme temperatures which has been found to be a major contributor to poor performance of RDTs in tropical countries, especially during transportation and storage [25, 29].

The pLDH-detecting RDTs produced a consistent level of parasite lactate when maintained at 4°C, but activity is reduced after cumulative exposure to temperatures likely to be encountered over a few months in a malaria-endemic area [30]. High humidity has also been found to rapidly degrade RDTs [31]. The environmental temperature in Maiduguri can reach up to 45°C and above, high temperature can degrade the RDT kits [29]. Maintaining a cold chain from the point of arrival of the RDTs into the country, storage and during transportation to point of care cannot be ascertained to be optimal. RDT technical problems can be from quality performance or assurance, test quality and accuracy, and the packaging of the kits [26]. RDTs are more expensive relative to microscopy [32] and also the intensity of test band varies with amount of antigen present at low parasite densities which may lead to reader variation in test results [7, 33].

However, the accuracy of microcopy test solely depends on the expertise of the laboratory scientist/technician in proper staining and microscopic identification of diagnostic features of malarial parasites' species. This is one of the reason why some private laboratories depends on RDT in the diagnosis of malaria, as they cannot afford to employ and maintain a skilled personnel/microscopist.

5. Conclusion

This study found that microscopy performed better than RDT in the diagnosis of malaria in this area. This confirm the importance of microscopic technique as a gold standard for the accurate diagnosis of malaria in tropical regions, especially where trained microscopists are present.

Compliance with ethical standards

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Disclosure of conflict of interest

We hereby declare that there is no conflicting/competing interest as authors of this paper.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study

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