UJMR, Volume 6 Number 2, December, 2021, pp 54 - 58 *ISSN: 2616 - 0668*
https://doi.org/10.47430/ujmr.2162.008
Received: 18th February, 2021 **Accepted:** 27th July, 2021

Comparative Analysis of Malaria Diagnosis Using Microscopy and Rapid Diagnostic Test (RDT) in Ijebu-Igbo North Local Government, Southwest Nigeria

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Abstract
Malaria remains one of the greatest concerns for the African population. To curb malaria, certain strategies need to be adopted like a quick diagnosis of the parasite within the human body, maintenance of proper environmental hygiene and use of other control methods. Diagnosis of malaria is often achieved using Microscopy, Rapid Diagnostic Test and Molecular Technique. This study aims to compare the effectiveness of Microscopy and Rapid Diagnostic Testing in diagnosing malaria infection in patients at the General Hospital, Ijebu-Igbo, Ogun State, Nigeria which also falls under a malaria-endemic region using 150 study participants. Blood samples were collected from study participants having malaria symptoms using ethylenediamine tetra-acetic acid (EDTA) container. The screening was done using microscopy method and Rapid Diagnostic Test. The data generated were analyzed using the Statistical Package for the Social Sciences (SPSS) version 19. The statistical parameter that was used for the analysis of the data was Pearson's Chi-Square Test, P at 0.05. The result shows the prevalence of malaria obtained through microscopic examination was 120 (80%) considerably more than RDT 54 (36%). These findings confirmed that microscopy is the gold standard in malaria diagnosis due to its high sensitivity, which allows it to detect parasites even at low counts. However, RDT has a specificity of 93.3% and a sensitivity of 92.2%, indicating that it is also effective when the parasite load is high. However, when compared to RDT kits for malaria diagnosis, microscopic analysis showed a higher sensitivity (100%); nevertheless, RDT may be a useful tool for rapid intervention to avoid the dangers associated with delayed diagnosis.
Keywords: Malaria, Prevalence, Diagnostic, Microscopic, RDT

INTRODUCTION

Malaria might be a severe illness caused by a protozoan parasite of the genus *Plasmodium* that is transmitted to people through the bites of infected female Anopheles mosquitoes (WHO, 2020). Five human *Plasmodium* species (*Plasmodium falciparum, P. vivax, P. ovale, P. knowlesi,* and *P. malariae*) cause malaria infection (White, 2008). The major complications are caused by *P. falciparum* and *P. vivax*, with *P. falciparum* being the more virulent (Geleta & Ketema, 2016). Malaria continues to pose a challenge to the African population with over 500 million infections yearly and over 2 million deaths annually (WHO, 2020). It remains a global burden tributary to morbidity and mortality particularly in children under 5 years of age (Tinashe *et al.*, 2018). Pregnant women andyoungsters underaged 5 years are the most foremost vulnerable group suffering from malaria (WHO, 2019). Malaria is also accounted for 67% of deaths in children (under five years old) worldwide (UNICEF, 2019). Despite the progress achieved towards malaria burden reduction, achieving elimination in more countries remains a challenge (Tinashe *et al.*, 2018). In Nigeria, malaria accounts for over 60% outpatient visits and 30% hospital admissions (Obimakinde *et al.*, 2018). The variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhoea and abdominal pain (Martins *et al.*, 2015).

Malaria must be recognized promptly to treat the patient in time and to stop further spread of infection in the community via local mosquitoes. It should be considered a potential medical emergency and will be treated accordingly (Hartjes, 2011). Delay in the diagnosis and treatment is a leading cause of death in malaria patients.

Although malaria can be suspected based on specific patient symptoms, however, for a definitive diagnosis to be made, laboratory tests must be carried out to detect the malaria parasites. Early detection amidst other factors will provide a drastic reduction in the degree of damage caused by malaria (Parker*et al.*, 2016). The accuracy of malaria diagnosis is vital for the effective management and control of malaria which will helps in preventing the incorrect usage of anti-malarial drugs that would lead to morbidity and mortality. There are several techniques used for diagnosis of malaria out of which includes the microscopy method, rapid diagnostic tests and molecular assay. Microscopy and rapid diagnostic tests (RDT), as well as clinical evidence, are the first choices for diagnosing malariain most malaria-endemic countries (Berzosa *et al.*, 2018).

Microscopic method of diagnosis of plasmodium involves the uutilization of stained blood smears. This method has been in use for a century and has been the main tool for the diagnosis of malaria in laboratories. This method is comparatively simple and requires less training (Wongsrichanalai *et al.*, 2014). Rapid Diagnostic Tests are based on the detection of antigens derived from malaria parasites in lysed blood, using immuno-chromatographic methods. Currently, this method is increasingly in use for diagnosis of malaria parasite because it is rapid, easy to use and does not require much training or special equipment (McMorrow *et al.*, 2011). However, there are limitations to the use of this method because the method is not sensitive to identify different species of *Plasmodium* and therefore, there is low sensitivity to detect parasitemia (de-Monbrison *et al.*, 2003).

The major means of effective disease management may be a quick and accurate diagnosis. As malaria-endemic countries move towards malaria elimination, there is a need for rapid and accurate diagnostic tools for malaria. This study aims to compare the performance of microscopic method and rapid diagnostic tests (RDT) in the diagnosis of malaria infection in patients at the General Hospital, Ijebu-Igbo, Ogun State, Nigeria

MATERIALS AND METHODS

Study Area

This study was conducted in Ijebu-Igbo, a town in Ijebu North Local Government, Ogun State, Nigeria. Ijebu North is one among the 20 Local Government Areas in Ogun State, South Western, Nigeria. It is located within the coordinates 6^0 57^1 N and40^1E. It has an area of 967 km^2 with a population of 284, 336 (2006 census), thereby making it one of the populated local government areas in the state. Ijebu-Igbo is bounded in the northern part by Oyo state, in the west by Ijebu-East Local Government Area, in the south by Ijebu North East Local Government Area, Odogbolu Local Government Area and Ijebu-Ode Local Government Area while it is bounded in the eastern part by Ikenne Local Government Area.

Samples collection

A total of One hundred and fifty (150) study participants were involved in the study between June 2019 and August 2019 comprising both male and female with the varying ages. With the use of needle and syringe, two to three millilitres of blood samples were collected from each patient intravenously. The blood samples were transferred directly into an Ethylene Diamine Tetra-acetic Acid (EDTA) container to prevent the blood samples from coagulation. The samples were taken to the laboratory for processing. The Blood sample collected from each patient was labelled with the identification details such as name, sex and age of the patient.

Rapid Diagnostic Test (RDT) for malaria

The kit used for this study was Carestart malaria pf (HRP- II) AG Test Kit which is one of the most commonly used kits and readily available in the Nigeria markets. This RDT is a qualitative immunochromatographic test that detects *P. falciparum* HRP-II, following the manufacturer's instructions. With the use of pipette in the kit, about 10 µl of blood was taken from the EDTA container and transferred into the sample well within the cassette. Three drops of buffer were added to the blood in the sample well after which was left for some minutes and allowed to flow to the result window on the cassette. After 15 minutes, the cassette was then checked for the appearance of coloured lines on the result window. The test was interpreted to be positive if a coloured line appeared at the control region of the cassette and the test region while the test was interpreted to be negative if only a single coloured line appeared at the control region of the cassette and non at the test region.

Microscopic diagnosis of malaria

A thin blood smear was used for microscopic screening of samples employing a standard protocol as used by (Pembele *et al.*, 2014).Blood films are made by placing a drop of blood on one end of a slide and using a spreader slide to disperse the blood over the slide's length. The aim is to get a region, called a monolayer, where the cells are spaced far enough apart to be counted and differentiated.

The slide was left to air dry, after which the blood was fixed to the slide by immersing it briefly in methanol. After fixation, the film was stained with Giemsa stain for 15 minutes. After 15 minutes, the stain was washed off rapidly with distilled water and allowed to dry. Two drops of immersion oil were added to the film and then view under the microscope at X100 objective lens for characteristics features of the plasmodium (Obimakinde *et al.*, 2018).

Statistical Analysis

The data generated were analyzed using the Statistical Package for the Social Sciences (SPSS) version 19. The statistical parameter that was used for the analysis of the data was Pearson's Chi-Square Test, *p* at 0.05.

RESULTS

Blood samples were collected and analysed from one hundred and fifty (150) study participants in which each was analysed and compared using two malaria diagnosis method; rapid diagnostic test and stained blood film microscopy. The analysis of the sample showed that 36% of the study population were positive for malaria when their samples were analysed using the RDT kit (Carestart) 80% was also equally found to be positive when their blood samples were analysed microscopically. The sensitivity and specificity of the RDT kit diagnosis were computed, the sensitivity value shows that the RDT method is highly sensitive in detecting malaria parasites only if the parasite load is higher in an individual.

Table 1: Prevalence of Malaria using Frequency Distribution of Frequency Distribution of Analyzed Result of Rapid Diagnostic Test Kit and Microscopy

Diagnostic method	Positive (%)	Negative (%)
RDT kit	**54 (36%)**	**96 (64%)**
Microscopy	**120 (80%)**	**30 (20%)**

Table 2: Comparison of the Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of the two diagnostic methods

Diagnostic Methods	Positive	Negative	Sensitivity	Specificity	PPV	NPV	T.A
RDT	54	96	92.2%	93.3%	97%	84.4%	92%
Microscopy	120	30	100%	100%	100%	100%	100%

Key:PPV= Positive Predictive Value; NPV= Negative Predictive Value; T.A= Test Accuracy

DISCUSSION

The prevalence of parasites obtained from this study using Microscopy and RDT ranges from 80 % to 36 %, this prevalence is similar to the report of Obimakinde *et al.*, (2018) that had microscopic screening as 71.43% and RDT as 65%. The high prevalence of 80% revealed by microscopic analysis and 36% prevalence recorded by Rapid Diagnostic Test disagrees with the report of Pembele *et al.*, (2015) where the microscopic analysis revealed 34.7% and Rapid Diagnostic Test had 44.44%.

In this study, the microscopic examination which is considered as the gold standard for malaria diagnosis and had a sensitivity of 100%, because it provides results that are consistently accurate and sufficiently timely, also it is cost-effective. This requires a comprehensive and functioning quality assurance programme (WHO, 2018). It was able to detect more parasites than RDT which had a sensitivity of 92.2%, this lower sensitivity is disadvantageous as it will impair control intervention since a fraction of the infected population will be left untreated especially if RDT is the only available diagnostic tool. This might have important implications in health, transmission, and possibly mortality. However, this study is in accordance with the work of Metoh *et al.*, (2020), that gave the sensitivity of Microscopy as 100%. This reveals that the Microscopy has higher sensitivity, which is an important factor for quantification of parasitemia. The study of Mfuh *et al.*, (2019) also confirmed that Microscopy predicted the presence of malaria parasite in 99% of the study participants making Microscopy a good "rule in" test for malaria, this suggests that a positive Microscopy result for malaria can be trusted (Mfuh *et al.*, 2019). During the study, the only species of Plasmodium found was *P. falciparum*. *P. falciparum* has been known to be the main cause of malaria in Africa,this is similar to the observation by Mordi & Morke, (2013). This study also corroborates the work of Yohanna *et al.*, 2019) where 71% of the test population were infected with *Plasmodium Falciparum*.

Malaria RDT kits are being increasingly adopted across endemic countries such as Nigeria to strengthen parasitological diagnosis and appropriate management. However, in this present study, malaria RDT could predict the presence of malaria parasite in 54 (36%) of the study participants making it a valuable

alternative in areas that do not have good resources for Microscopy.

This study also confirmed the use of malaria RDTs as recommended by the World Health Organization (WHO) when reliable microscopy is not available (Azikwe *et al.*, 2012). Therefore, Symptomatic Diagnosis and malaria RDT can be used to improve the quality of care by ensuring the appropriate treatment of confirmed malaria cases. It is therefore not sufficiently sensitive for mass screening programmes as recommended by the WHO. The RDT has also been reported to give false negative results even at a higher level of parasites.Therefore, if severe malaria is suspected, negative RDTs should be double-checked and confirmed by Microscopy (Leke, 2009).

CONCLUSION AND RECOMMENDATIONS

This study has compared the effectiveness of two diagnostic methods for screening malaria in the study area. The Rapid Diagnostic Test (RDT) and Microscopy examination revealed the different prevalence rates of the parasites. However, Microscopy examination had higher prevalence rate compared to the Rapid Diagnostic Test, although the Microscopic Test requires a very standard laboratory with well-trained personnel. Likewise, there was significant difference in the prevalence rate of the parasites in rapid diagnostic test (RDT) compared to microscopic examination. The rapid diagnostic test (RDT) had a lower detection of the parasites which could probably be as a result of its inability to detect parasites when counts are very low but it is very quick and convenient to use. However, based on this finding, the rapid diagnostic test kits can be used safely in place of microscopic. As such, the use of microscopic method for malaria screening is effective and highly recommended due to its sensitivity, cheap cost and low technicality.

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