Original

A Cross- Sectional Study on the Comparative Analysis of Diagnostic Methods and Malaria Distribution in Febrile Patients Attending a Multi-System Hospital in Ado-Ekiti, Ekiti State, Nigeria

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Abstract

Background: Malaria remains a significant global health concern despite improvements in treatment and vector control. This study aimed to assess the prevalence of Plasmodium falciparum infection and compare the diagnostic accuracy of microscopy versus rapid diagnostic tests (RDTs) in febrile patients at Afe Babalola Multi-System Hospital in Ado-Ekiti, Ekiti State, Nigeria.

Method: A total of 150 samples of were enrolled using purposive sampling method between March and April, 2024 and analyzed for malaria parasites using RDTs and microscopy.

Results: The study included 92 males (61.3%) and 58 females (38.7%) with ages ranging from 14 years and older. The age group 18-24 years had the highest proportion of cases (34%), while those aged 60 and above had the lowest (3.3%). A majority of patients (71%) were from urban areas. Common symptoms included headache (34.7%), body pain (29.3%), fatigue (28.7%), and fever (24.7%), while chills (4.7%) and anemia (3.3%) were less frequently reported. Diagnostic results showed that 96% of samples tested positive for *P. falciparum* by microscopy, compared to 88% using the RDT.

Conclusion: The findings underscore higher prevalence in urban areas and higher malaria burden in young adults, particularly males, suggesting that factors such as poor sanitation, informal housing, stagnant water sources, and population density may contribute to transmission in cities, also gender disparities in malaria incidence may stem from differences in exposure risks, access to healthcare, or biological susceptibility. The study also highlights the value of combining microscopy and antigen-based tests for reliable diagnosis, especially in urgent care settings.

Keyword: *Plasmodium falciparum*, microscopy, rapid diagnostic test, febrile illness, Nigeria.

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INTRODUCTION

Despite recent advancements in chemotherapeutics and vector control measures, malaria has caused an unacceptably high rate of morbidity and mortality among people worldwide for more than a century.[1] In particular, a number of obstacles, including parasite resistance and zoonotic instances, are making it difficult for the malaria-endemic nations to eradicate the disease at this time. Malaria incidence increased from 221 million cases in 2019 to 247 million cases in 2020, according to the World Health Organization's most recent study [2]. This is a significantly greater case burden than in 2015, when numbers were notably declining from 2000. Furthermore, since 2015, the number of malaria infections and fatalities has not significantly decreased [2]. With an estimated 55% of malaria cases in West Africa in 2022, Nigeria is responsible for the largest proportion of the global malaria burden, accounting for 27% of estimated malaria cases worldwide and 31% of estimated deaths, according to the 2022 World Malaria Report [3].

The protozoan parasite Plasmodium spp. causes malaria, an infectious disease that is spread by female Anopheles mosquitoes carrying Plasmodium infections during bloodmeal. Shivering, body aches, abdominal pain, fever paroxysms, and other flu-like symptoms are common signs of malaria [4]. Acute respiratory distress syndrome (ARDS), placental malaria, cerebral malaria, anemia, liver failure, and kidney failure are among other clinical symptoms associated with severe malaria. In particular, in high-risk individuals like infants, young children, pregnant women and their unborn children, older adults, and visitors from non-endemic malaria countries, these symptoms can be fatal if not identified and treated promptly [5,6,7]

Malaria is diagnosed using a variety of laboratory techniques, such as molecular approaches, immunological testing, and microscopy [8]. Since it can detect malaria parasites in blood samples directly, microscopic analysis of blood films is still the gold standard for diagnosing malaria [9] This technique looks for Plasmodium species in blood by staining it and looking at it under a microscope. This is because it can identify the species of malaria parasite and measure parasitemia, microscopy is generally considered the most trustworthy method [10]. However, in remote or resource-constrained areas, microscopy may not be feasible due to the need for highly qualified staff and substantial resources, such power supply.

Rapid diagnostic tests (RDTs), on the other hand, have grown in popularity because of their practicality and simplicity of use. RDTs rely on the identification of particular antigens from malaria parasites, including parasite lactate dehydrogenase, aldolase, and histidine rich protein 2 (HRP2) [11]. These tests, which usually produce findings in 5 to 20 minutes, identify these antigens in lysed blood samples using immunochromatographic techniques.
RDTs are beneficial due to their ease of use, lack of electricity requirements, and interpretable outcomes [12,13]. Additionally, commercial RDT kits are widely accessible and frequently employ dipsticks that include monoclonal antibodies that specifically target parasite antigens. These test kits come in a variety of formats, such as cassettes, dipsticks, and card-flaps, offering a selection to meet diverse diagnostic requirements [14]
The aim of this study is to investigate *P. falciparum* and to compare the effectiveness of microscopy and rapid diagnostic techniques in detecting malaria among febrile patients attending Afe Babalola Multi-System Hospital, Ado-Ekiti, Ekiti State, Nigeria. This comparison will provide valuable insights into the reliability, speed, and practicality of these diagnostic methods, especially in settings with limited resources. By evaluating these methods, the study aims to contribute to improving malaria diagnosis and, ultimately, the management of this deadly disease in Nigeria and similar regions.

MATERIALS AND METHODS

Study area and sample collection

The study was conducted at Afe Babalola University Multi-System Hospital in Ado-Ekiti, Ekiti State, Nigeria. Ado-Ekiti is the capital city of Ekiti State, Nigeria and the headquarters of the Ekiti Central senatorial district, in the southwest, of Nigeria. The total land area is 293 km2. Ado Ekiti is the Ekiti state capital and a Local Government Headquarter in one of the sixteen Local Government Areas in Ekiti state. It lies within Latitude 7°10' and 7°45' north of the Equator and Longitudes 5°10' and 5°28' east of the Greenwich meridian [15].

Ethical approval

Ethical approval for the study was obtained from the ethical committee of Afe Babalola University Multi-

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System Hospital in Ado-Ekiti, Ekiti State, Nigeria with ethical approval number AB/EC/24/03/063.

Inclusion criteria

(i) Patients presenting with fever (temperature ≥ 38°C) from age range 14 years and older at the study site during the study period.
(ii) Patients who consent to participate in the study.

Exclusion criteria

(i) Patients who have received antimalarial treatment within the last 2 weeks prior to presentation.
(ii) Patients with known chronic illnesses that could cause fever
(iii) Patients who decline to participate or do not provide informed consent.

Sample size

The study conveniently considered 150 patients between March and April, 2024 and questionnaires were used to get the demographic information of the patients. The sample size was determined using the formula for estimating the minimum number of samples needed to obtain reliable results in studies of disease prevalence. The sample size was calculated based on the expected prevalence of malaria and the desired confidence level. The formula below was used to estimate the required sample size.

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

Z - value corresponding to the desired confidence level, which is 1.96 for a 95% confidence
Level.
P - estimated prevalence rate of malaria = 12.5% [16]
d - is the margin of error, set at 0.05 reflecting the desired level of precision in the estimate.

Sample collection and analysis

Venous blood was collected from the patients using a 2 ml ethylene diamine tetra acetic acid vacutainer. A drop of blood was placed on a microscope slide and spread to cover an area of about 1 square centimetre (1 cm^2) to create a thick blood smear for microscopy. The film was spread thin enough so that it appeared transparent. It was airdried, and care was taken not to fix the thick smear and subsequently stained with Giemsa staining technique. The smear was air-dried and examined using high power magnification. Before a slide was declared negative for microscopy, a minimum of 200 microscopic fields were examined at a magnification of X1000 with oil immersion optics.

The Rapid Diagnostic Test (RDT) utilized in this study was the SD Bioline Malaria Ag P.f. Histidine-Rich Protein II (HRP-II) with Lot: PF2301. The test was conducted strictly following the manufacturer's instructions to ensure accuracy and consistency.

Statistical analysis

Statistical analysis of the data was conducted to evaluate the significance of variables, with frequencies and statistics employed to use for variables like gender, location, age group and symptoms while Chi-square test was employed to compare between RDT and microscopy, urban and rural prevalence and male and female infection rates. Diagnostic accuracy measures such as sensitivity and specificity were also relevant in this study to confirm the technique which is the gold standard.

RESULTS

Through microscopy and immunoassay (an antigen-based test), out of the 150 febrile patients' samples collected 132 were positive for malaria parasite using RDT while 144 sample were positive using microscopy. Figure 1 shows sex-based patient distribution which revealed that there were 92 (61.3%) men and 58 (38.7%) women, all of whom were at least 14 years old. Both adults and adolescents were included in the data collection, as evidenced by the patients' age range. The distribution by age in Figure 2 revealed that the highest percentage, 34%, was among those aged 18 to 24 and the lowest percentage, 3.3%, was among those aged 60 and above. According to Figure 3, there were more people living in urban areas (106, or 71%) than in rural regions (44, or 29%). Patients' symptoms were shown in Figure 4, with headache, body aches, exhaustion, and fever ranking highest at 52 (34.7%), 44 (29.3%), 43 (28.7%), and 37 (24.7%). The patients' least common symptoms were chills 7 (4.7%) and anemia 5 (3.3%). The majority of them have many symptoms. Table 1 summarizes the percentage of positive and negative results from microscopic and RDT antigen screening. Plasmodium falciparum was found in 144 (96%) of the samples examined under a microscope in the blood films, whereas 132 (88%) of the samples tested positive for the antigen-based test.

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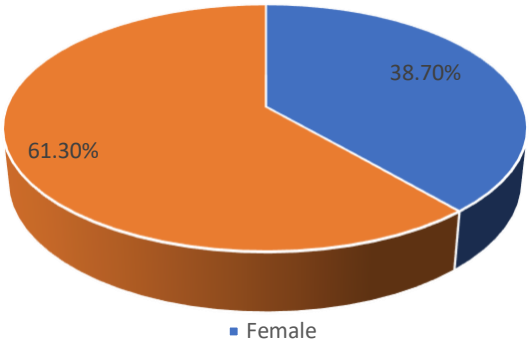

Figure 1: Distribution according to gender

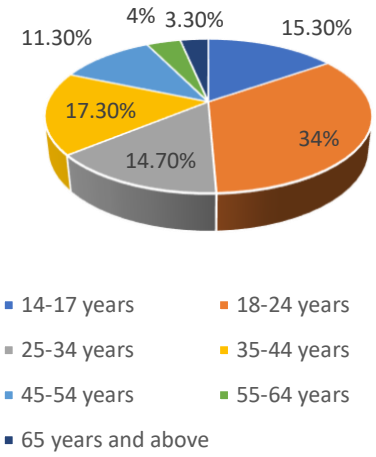

Figure 2: Distribution according to age

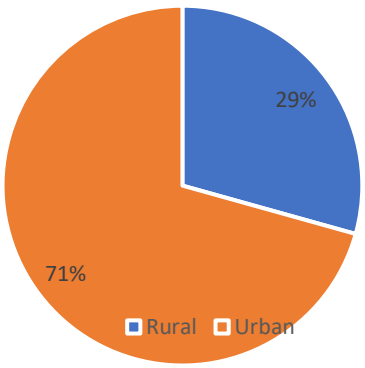

Figure 3: Distribution according to location

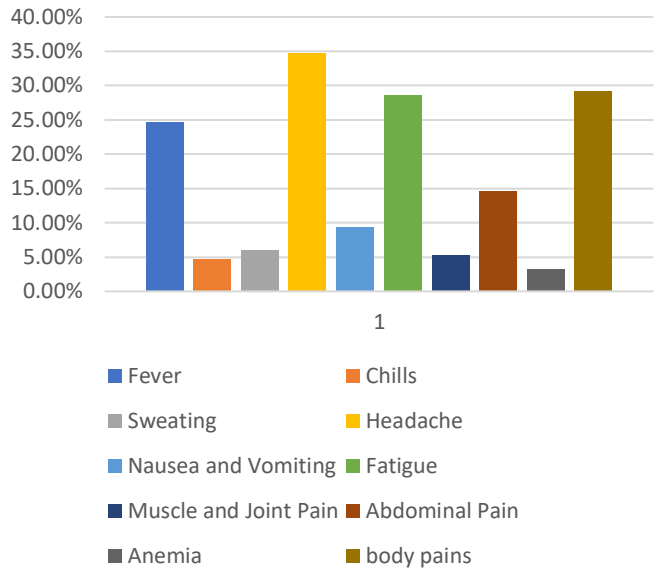

Figure 4: Bar chart representing symptoms

Table 1: Summary of positive/negative figures

Status	RDT Antigen Test	Microscopy
Negative	18	6
Positive	132	144
Total	150	150
Percentage positive	88%	96%

DISCUSSION

This study used both microscopy and an antigen-based immunoassay (rapid diagnostic test, RDT) to examine the distribution and features of febrile patients with malaria, specifically *Plasmodium falciparum*-caused malaria. The study offers important information about the frequency of malaria, the characteristics of those who are afflicted, the symptoms they display, and the precision of various diagnostic techniques. An in-depth discussion based on the study's findings is provided below.

The findings showed that there were 92 male patients (61.3%) and 58 female patients (38.7%) with a diagnosis of Plasmodium falciparum malaria. This implies that males in the studied population had a higher incidence of malaria. This gender gap may be caused by a number of things. Males exhibited a greater frequency of malaria

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(95.0%) than females (93.3%), according to a prior study conducted in Ekiti State by Awosolu *et al.* (2021) [17]. Males may be more susceptible to malaria because of lifestyle or work-related variables. For instance, men could be more likely to work outside or partake in other outdoor activities in regions where malaria is widespread, which could make them more vulnerable to mosquito exposure. Yet, the gender gap observed in this study might potentially be unique to the area and change based on the region's more general epidemiological characteristics.

The patients included both adolescents and adults, with ages ranging from 14 years of age and up. According to the study, 34% of patients were between the ages of 18 and 24, which was the age group with the highest percentage of malaria cases. The elevated risk of malaria in young adults, who are frequently more socially engaged and participate in outdoor activities that raise their risk of exposure, may be reflected in this study. This contrasts with research conducted in the same state by Awosolu *et al.* (2021), which found that the age group <10 years had the lowest malaria parasite prevalence (86.0%) and the age group 51 to 60 years had the highest prevalence (100%) of malaria parasites [17]. A notable percentage of malaria cases (15.3%) occur in the 14–17 age group, which may be related to school-age children in malaria-endemic areas. These children are frequently exposed to malaria while playing or going on walks outside in places where the mosquito vector is common. However, the age group of 60 and above had the lowest rate of infections (3.3%), which may indicate that older people have taken extra care to avoid malaria or have developed partial immunity over their lives. But this age group might also be more inclined to get help when they feel sick, which could result in early identification and treatment, shortening the infection's duration and severity.

Contrary to the widely held belief that rural areas are more susceptible to malaria because of things like less sanitation and more mosquito breeding grounds, the study's results show an unexpected trend. A larger percentage of malaria cases (71%) were identified in urban areas. However, according to data from the US President's Malaria Initiative in Nigeria (2023), the prevalence of malaria is 2.4 times higher in rural regions than in urban ones (31% vs. 13%)[18]. This disparity between the study's results and the national prevalence data may indicate that additional factors, such as higher population density, different travel habits, or variations in healthcare reporting and access, are at work in urban regions. More people may seek medical care for feverish illnesses in urban locations, which could result in more cases being diagnosed. To determine the reasons behind this disparity, more research would be required.

Patients in this study reported symptoms that are similar to those commonly seen in *Plasmodium falciparum* malaria, with fever, headache, bodily discomfort, and exhaustion being the most prevalent. It is interesting to note that anemia and chills, two well-known signs of malaria, were reported by fewer patients in this study. There are significant variations from the results of a study conducted in Cameroon by Hodson *et al.* (2022). A greater proportion of patients in the Cameroonian trial had chills (53.5%), headache (79.4%), exhaustion (92.4%), and fever (97.2%)[19]. These discrepancies can be the result of differences in the two populations' access to healthcare, reporting practices, or the severity of the condition. Nonetheless, the primary symptoms fever, headache, and exhaustion remain constant, underscoring their significance as crucial markers for the diagnosis of malaria. It is important to note that, as is common with malaria, the majority of patients had several symptoms. This is because malaria infections can exhibit a variety of symptoms, it can be difficult to make a clinical diagnosis based just on symptoms without test confirmation.

This study employed two diagnostic approaches to confirm *Plasmodium falciparum* infection: microscopy and antigen-based Rapid Diagnostic Tests (RDTs). RDTs detected the parasite in 88% of the samples, whereas microscopy was very successful in detecting it in 96% of the samples. However, a study by Comfort et al. (2024) found that just 14% of samples tested positive by microscopy and 4.7% by RDTs, indicating substantially lower detection rates [20]. In contrast, Azikiwe et al. (2012) found that 59% of microscopy tests were positive and 64% of RDT tests were positive [19]. These differences point to the increased sensitivity of microscopy, which is still the gold standard for diagnosing malaria, particularly in settings with adequate resources, and imply that diagnostic accuracy varies throughout investigations. However, because RDTs offer a quicker, simpler, and more accessible diagnostic alternative especially in rural locations or settings with limited access to laboratory infrastructure they are still useful for quick, field-based diagnosis, especially in resource-constrained areas. Despite having a somewhat lower detection rate (88%), the RDT is incredibly useful in situations where time and resources are limited. The quality of the blood smear, the degree of parasitemia, or the timing of the infection in relation to the patient's presentation could all be contributing factors to the 8% difference between the two approaches.

Conclusion

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In conclusion, this study has provided valuable insights into the distribution, symptoms, and has compared diagnosis between RDT and microscopic examination of *Plasmodium falciparum* among febrile patients at Afe Babalola Multi-System Hospital in Ado-Ekiti, Ekiti State, Nigeria. The study confirmed that malaria continues to significantly impact young adults, particularly those in the 18-24 age group, and that males are more affected than females. The finding that the majority of patients reside in urban areas, a trend not commonly observed in many malaria-endemic regions, warrants further investigation into the factors contributing to this discrepancy.

The study also highlighted the importance of accurate and timely diagnosis for effective malaria management. The results indicated that microscopy remains the gold standard for detecting *P. falciparum*, with a higher sensitivity compared to the RDT. However, the RDT is still a useful tool, particularly in settings where rapid diagnosis is crucial. Combining both methods can improve diagnostic accuracy and ensure better outcomes, especially in emergency and resource-limited environments.

Additionally, the symptoms of headache, body pain, fatigue, and fever were most commonly reported, aligning with typical malaria symptoms, but the lower incidence of chills and anemia suggests that the clinical presentation of malaria may vary, highlighting the need for comprehensive symptom assessment in diagnosis.

Future research should focus on understanding the factors contributing to the higher malaria prevalence in urban areas, exploring potential differences in vector control effectiveness, sanitation, and healthcare access. Furthermore, gender disparities in malaria incidence and the impact of socioeconomic factors should be addressed to inform more targeted interventions and public health strategies. This study reinforces the need for continued efforts in malaria prevention, diagnosis, and treatment to reduce the burden of this preventable disease.

Limitation

Due to paucity of funds, we could not more tertiary healthcare institutions in the state.

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