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Assessment of the Different Methods of Diagnosis of Malaria Parasite Among Children in Port Harcourt Metropolis

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Abstract **Original Research Article**

Malaria affects millions of lives annually, and it is considered as one of the major global health challenges**.** *Plasmodium* species are responsible for malaria infection, and are transmitted through the bite of infected female Anopheles mosquitoes. Over 90% of malaria related death world-wide is caused by *Plasmodium falciparum.* This cross sectional study was carried out to determine the different methods of diagnosis of malaria parasite among children within Port Harcourt metropolis, Rivers state Nigeria. A total of 264 children (116) male and (148) female were recruited for the study. Venous blood collected from the subjects using Ethylene Diamine Tetra Acetic Acid (EDTA) container were examined using Giemsa stained thick and thin blood film and malaria Rapid Diagnostic Test (RDT) kits. The result revealed an overall prevalence of 12.5% for microscopy, while RDT showed 8.7% prevalence. Children under the age of 5 had a higher prevalence by microscopy (13.04%) compared to RDT (8.07%). For age between 5-10, microscopy also detected more cases (11.65%) than RDT (9.71%). The P-value (0.7385) indicates there is no significant difference between age groups and malaria infection. Microscopy showed a slightly higher infection rates in females (12.84%) than males (12.07%), while RDT results are similar with slightly higher infection rates in females (8.78%) than males (8.62%). The P-value (0.6616) indicates there is no significant difference between gender and malaria prevalence. The diagnostic comparison of Microscopy and RDT results in the study showed that Microscopy had a high sensitivity of 94.29%, while RDT's sensitivity is lower at 67.65%. Both tests have perfect specificity (100%). The positive predictive value (PPV) is 100% for microscopy but only 69.7% for RDT. Overall, microscopy outperforms RDT in detecting malaria accurately, especially in identifying positives. The higher infection rate found in children under five years old highlights that they are the most vulnerable group and should be a major focus for prevention and control efforts.

Keywords: Malaria prevalence, Plasmodium falciparum, microscopy and rapid diagnostic test (RDT), pediatric malaria infection.

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CHAPTER 1

INTRODUCTION

1.1 Background to the Study

Malaria affects millions of lives annually, and it is considered as one of the major global health challenges, predominantly in Non-Mediterranean Africa, South Asia, and parts of Latin America (5). In the year 2020, 241 million malaria related cases were recorded by the World Health Organization (WHO), with 627,000 death recorded in 2020 alone. Young children especially those under five, are more prone to contracting malaria due to their vulnerable immune system (10). *Plasmodium* species are responsible for malaria infection, and are transmitted through the bite of infected female Anopheles mosquitoes. Over 120 *plasmodium* species exist, but five has been found to cause malaria in human, namely, *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium ovale*, *Plasmodium malariae,* and *Plasmodium knowlesi*. Over 90% of malaria related death world-wide is caused by *Plasmodium falciparum* (8). In Africa, Western Pacific, Eastern Mediterranean, and south-East Asia, *Plasmodium falciparum* is a major etiological factor for the majority of malaria cases, accountable for over 99%, 71.9%, 69%, and 62.8% infections respectively (15). Evidence shows that *Plasmodium vivax* often associated with uncomplicated malaria can also lead to severe illness. *Plasmodium malariae* and *ovale*, causes uncomplicated malaria, with *Plasmodium knowlesi*, a zoonotic parasite which are transmitted from primate to humans responsible for severe malaria (2). *Plasmodium* has an intricate life cycle, involving human and mosquito. In human the parasite evolves through the liver and blood stages, where it invades the red blood cells, triggering Malaria symptoms like anaemia, chills, and fever (13).

The equatorial region which spans across Africa, Asia, and Latin America, exhibits high concentration of malaria prevalence. The abundant rainfall in the tropical and subtropical regions results in the formation of stagnant water bodies, such as pond, lakes, and swamps. Alongside, the warm temperature in these regions, which ranges from 20℃ to 30℃, facilitates the rapid growth and development of Anopheles mosquito larva. malaria exacts a devastating toll on children under five years of age and pregnant women, while also inflicting significant economic losses, estimated at $12 billion in Africa, and reducing economic growth by 1.3%. in 2018, a

1.2 Statement of the Problem

Plasmodium species are responsible for malaria infection, and are transmitted through the bite of infected female Anopheles mosquitoes. Over 90% of malaria related death world-wide is caused by *Plasmodium falciparum* (12). In Africa, Western Pacific, Eastern Mediterranean, and south-East Asia, *Plasmodium falciparum* is a major etiological factor for the majority of malaria cases, accountable for over 99%, 71.9%, 69%, and 62.8% infections respectively (Varo *et al.*, 2020). Malaria affects millions of lives annually, and it is considered as one of the major global health challenges, predominantly in Non-Mediterranean Africa, South Asia, and parts of Latin America (14). In the year 2020, 241 million malaria related cases were recorded by the World Health Organization (WHO), with 627,000 death recorded in 2020 alone. Young children especially those under five, and pregnant women are more prone to contracting malaria due to their vulnerable immune system (1). The abundant rainfall in the tropical and subtropical regions results in the formation of stagnant water bodies, alongside, the warm temperature in these regions, which ranges from 20℃ to 30℃, facilitates the rapid growth and development of Anopheles mosquito larva. Malaria exacts a devastating toll on children under five years of age and pregnant women, resulting into High fever, headache, shaking chills, anaemia, spontaneous abortion, and in chronic untreated cases could lead to death (15).

1.3 Justification of the Study

Malaria affects millions of lives annually, and it is considered as one of the major global health

challenges. In the year 2020, 241 million malaria related cases were recorded by the World Health Organization (WHO), with 627,000 death recorded in 2020 alone. Children, are more prone to contracting malaria due to their vulnerable immune system. Due to the climatic and environmental conditions of different locations in Port Harcourt, there is the need for timely diagnosis so as to enable early treatments. This is very crucial for the in harnessing effective malarial control.

1.4 Aim of the study

The aim of this study was to determine the different methods of diagnosis of malaria parasite among children in Port Harcourt.

CHAPTER 2

MATERIAL AND METHODOLOGY

2.1 Study Area and Study Population

This study is conducted in Port Harcourt, located in Southern Nigeria, within Latitude 4º 42' 00"to 4º 57' 03" North and Longitude 6º 53' 11" to 7º 8' 49" East, occupying an area of approximately 369 km². It is the capital city of Rivers State consisting of two Local Government Areas (LGAs) which are Obio/Akpor and Port Harcourt LGAs. Port Harcourt, a blessed and lively city in Nigeria known for its oil and commercial activities, attracting diverse personalities from all parts of the country, hence, increasing the population of the state to about 1,005,904 (11). Children, are more prone to contracting malaria due to their vulnerable immune system.

For this cross-sectional study, a total of 264 children school were selected by stratified random sampling within Port Harcourt metropolis.

2.2 Sample Size Determination

The minimum sample size was calculated using the Cochran's sample size formula (Cochran, 1977) which is shown below (Eze *et al.,* 2021);

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

Where;

N = Minimum sample size

Z = Z-value corresponding to 95% confidence level set at 1.96

p = Prevalence of malaria from a recent study within the study area = 12.3% = 0.123

1 – p = Proportion of the population that does not possess the characteristic = 1 – 0.123 = 0.

d = Desired precision (sample error), 5% =0.05

The population size was increased to 264 subjects.

2.3 Selection Criteria

2.3.1 Inclusive Criteria

1 Subject who resides within Port Harcourt metropolis
2 Children not on malaria medication 2 weeks before sample collection

2.3.2 Exclusive Criteria

1 Non-resident of Port Harcourt
2 Children who have been on antimalaria medication 2 weeks before sample collection.

2.4 Ethical Approval: Was sought at the River State University Teaching Hospital by the ethical committee

2.5 Data Collection

Blood samples were collected from children within school age within Port Harcourt through venipuncture. whereby touniquette was tied around the upper arm, which increases the blood pressure in the veins. The selected puncture sight was thoroughly cleaned using cotton wool moistened with methylated spirit, before using the syringe to draw two milliliters (2ml) of

blood. Rapid Diagnostic Test was carried out immediately at the site of sample collection using Care Start™ Malaria HRP2 (Pf) kits according to the manufacturer's instruction and the result recorded. Venous blood obtain was then transferred into an Ethylene Diamine Tetra-acetic Acid (EDTA) sample bottle to prevent coagulation of the sample, then each bottle was assigned a specific code.

2.6 Laboratory Analysis for Malaria Parasite Identification

2.6.1 Malarial Rapid Diagnostic Test (RDT)

Rapid Diagnostic Test was carried out for each sample using Rapid Test kits for *Plasmodium falciparum* malaria manufactured by Arkray. The foil pouch containing the cassette was torn at the notch, the cassette was then brought out and placed on a flat surface. 5µL of whole blood were added into the sample well, after which 3 drops of clearing buffer was added to the well containing the whole blood. The sample was then allowed to migrate. Result was read after 20 minutes (Warhurst & Williams, 2017).

2.6.2 Thick film Method: The thick film was prepared according to (Cheesbrough, 2010). A drop of blood was placed on a clean grease-free glass slide and spread to cover up to 15 mm in diameter, after which it was allowed to airdry. The unfixed film was then stained for 15 minutes using 1:20 dilution of Giemsa solution. The slide was then gently rinsed using few drops of distilled water and then allowed to air dry. The slide was then examined under the microscope using ×100 oil immersion objective lens to detect the presence of *plasmodium.* The film was considered to be positive for malaria parasite if f the ring form of trophozoites or any other blood stage of erythrocyte schizogony was detected the film is said to be positive. If no parasites are seen after scanning 100 fields or more, this means that the result is negative (Warhurst & Williams, 2017).

2.6.3 Thin film method: The thin film was prepared according to (Cheesbrough, 2010). A drop of blood was placed on a clean grease free glass slide. The glass spreader was placed on the glass slide such that it makes contact with the drop of blood which runs along its edge. At an angle of 45, the spreader was pushed away from the drop of blood such that it forms a head and a tail. The film was allowed to airdry and then fixed with absolute methanol for 2 minutes, after which it was stained for 15 minutes with 1:20 dilution of Giemsa stain. The slides were then rinsed in running water. It was then dried and examined under the microscope using the x100 oil immersion objective lens for *plasmodium* species identification (Warhurst & Williams, 2017).

2.7 Statistical Analysis

Data obtained from this study was analyzed using Graph pad prism statistical software, version 8.0 by San Diego, California, United State of America. Descriptive statistics of tables and percentages were used for the categorical variables. Inferential statistics of data were tested using Chi-square at a significance level of $P < 0.05$

CHAPTER 3

RESULTS

Table 3.1 showed the prevalence of malaria in the study population according to age group. Based on overall prevalence, microscopy identified 12.5% infected, while RDT found 8.7% infected. Children under 5 had a higher prevalence by microscopy (13.04%) compared to RDT (8.07%). For ages 5-10, microscopy also detected more cases (11.65%) than RDT (9.71%). The P-value (0.7385) indicates there is no significant difference between age groups and malaria infection.

Table 3.1: Prevalence of Malaria in the Population According to Age Group

Age Group	Total No. Examined	Total No. From Microscopy Infected n (%)	Total No. From RDT Infected n (%)	Chi Square (χ^2)	P-value
< 5 years	161	21 (13.04)	13 (8.07)		
5-10 years	103	12 (11.65)	10 (9.71)		
Total	**264**	**33 (12.50)**	**23 (8.71)**	0.1114	0.7385

Table 3.2 below compares microscopy and RDT for diagnosing malaria. Microscopy shows a high sensitivity of 94.29%, while RDT's sensitivity is lower at 67.65%. Both tests have perfect specificity (100%). The positive predictive value (PPV) is 100% for microscopy but only 69.7% for RDT. Overall, microscopy outperforms RDT in detecting malaria accurately, especially in identifying positives.

Table 3.2 Diagnostic Comparison of Microscopy and RDT Results in the Study

Criteria	Microscopy (%)	RDT (%)
Sensitivity	94.29	67.65
Specificity	100	100
Positive Predictive Value (PPV)	100	69.70

Negative Predictive Value (NPV)	99.14	95.45

CHAPTER 4

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

Based on the findings from this study in Port Harcourt, where microscopy showed a higher prevalence of malaria (12.5% overall) than rapid diagnostic tests (RDTs) (8.7% overall), and where young children under five had the highest infection rates, the results align with a broader body of previous research, while also highlighting specific challenges regarding malaria within Nigeria. When compared with other recent studies in Nigeria, a consistent pattern emerges. For instance, a 2021 study conducted in a high-transmission area of Nigeria similarly reported that microscopy detected a higher proportion of malaria cases compared to some RDTs, particularly in children, who are more vulnerable due to developing immunity (Onyekwere *et al*., 2021). This difference is often attributed to the superior sensitivity of microscopy in detecting low-level parasitemia that RDTs, which rely on a specific protein (HRP-2), cannot detect, especially if parasite densities are below the test's detection threshold or in cases of gene deletions affecting the protein.

However, contrasting findings have been reported in other African countries with different malaria epidemiology. A 2023 study in Ethiopia, a region with lower and more unstable transmission, found that certain newer-generation RDTs performed with sensitivity comparable to, or even exceeding, that of routine microscopy when conducted in field conditions (Teshome *et al*., 2023). This contrast suggests that the performance gap between the two diagnostic tools may be narrower in low-transmission settings or when high-quality RDTs are used, whereas in high-transmission settings like Nigeria, the burden of low-density infections is greater, favoring microscopy's detection capability. The higher prevalence in children under five found in the Port Harcourt study is a universal finding, supported by a 2022 multi-country analysis across sub-Saharan Africa which confirmed that this age group bears the greatest burden of malaria infection and disease (5). The non-significant P-value (0.7385) indicating no link between specific age groups (above and under five) and infection in the Port Harcourt study is somewhat unusual and contrasts with a 2024 report from Ghana which demonstrated a statistically significant decline in prevalence and clinical cases as children grow older and acquire partial immunity, making the Port Harcourt finding an area that might require further investigation into local transmission dynamics or study population characteristics (11).

Furthermore, in this study, microscopy showed a high sensitivity of 94.29%, meaning it detects most true positives, while RDT's sensitivity is lower at 67.65%. Both diagnostic methods had perfect specificity (100%), so they correctly identify all true negatives. The positive predictive value (PPV) is 100% for microscopy but only 69.7% for RDT, indicating microscopy's better accuracy in confirming positive cases. Overall, microscopy outperformed RDT in detecting malaria accurately, especially in identifying positives. This implies that while RDTs are useful for quick treatment, microscopy remains a more sensitive method for detecting the infection, especially cases with lower levels of the parasite. it can be concluded that the choice of diagnostic tool is very important for accurately measuring malaria in this community. Therefore, this study's findings reinforce that in high-transmission areas of Nigeria, microscopy remains a crucial, more sensitive tool for accurate surveillance and

diagnosis, particularly for vulnerable groups like young children, even as RDTs continue to be indispensable for rapid case management in resource-limited clinics.

5.2 Conclusion

In conclusion, This study showed that microscopy method (12.5) had a higher prevalence than RDT method (8.5) and also the microscopy method had a higher sensitivity rate than RDT (94.29, 67.25 respectively). The higher infection rate found in children under five years old highlights that they are the most vulnerable group and should be a major focus for prevention and control efforts. To effectively fight malaria in Port Harcourt, programs must continue to use reliable diagnostic methods like microscopy for surveys and must protect young children above all others. Overall, the findings of this study is crucial for creating successful and efficient malaria control strategies tailored to the specific needs of Port Harcourt.

4.3 CONTRIBUTION TO KNOWLEDGE

Malaria parasitic infection which an endemic disease in this part of the world and has been a recurrent disease. The study was able to provide the prevalence rate of malaria infection based on the methods used in this locality as 12.5% by microscopic method and 8.5% by RDT.

4.4 Recommendations

1. Given its higher sensitivity and specificity, microscopy should remain the primary diagnostic method for malaria. It provides accurate results, enabling timely and appropriate treatment.

2. Investment should be made in training and continuous professional development for laboratory personnel to ensure they maintain high standards in microscopy techniques and interpretation.

3. In situations where microscopy is unavailable or impractical, rapid diagnostic test kits (RDTs) could serve as an alternative for initial screening. However, positive results should be confirmed with microscopy for more reliable diagnosis.

4. Regular quality assurance checks for both microscopy and RDTs to ensure consistent and reliable diagnostic performance should be implemented.

DISCLAIMER

All products used for this research are commonly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products used because we intend to use the products only for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Individuals recruited as participants in this study were those who gave verbal informed consent. Ethical approval was obtained from Rivers State Ministry of Health, Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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