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Comparative Malaria Diagnosis Between Microscopy and Rapid Diagnostic Tests Among Pregnant Women Attending Ahmad Sani Yariman Bakura Specialist Hospital in North-Western, Nigeria

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Abstract

- *Background:*

Malaria remains a major public health problem in Nigeria, particularly among pregnant women, where it contributes significantly to maternal and fetal morbidity and mortality. Accurate and timely diagnosis is essential for effective treatment and control of the disease.

- *Aim:*

This study compared the diagnostic performance of microscopy and rapid diagnostic tests (RDTs) among pregnant women attending ANC at YBSH Gusau, Zamfara State, Nigeria.

- *Materials and Methods:*

A hospital-based cross-sectional study was conducted among 250 pregnant women. Venous blood samples were collected and examined using microscopy and two malaria rapid diagnostic tests kits (SD Bioline and Akray). Microscopy served as the gold standard for evaluating the performance of the rapid diagnostic tests. Data were analyzed using descriptive statistics, and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the rapid diagnostic tests were determined.

- *Results:*

Microscopy detected malaria parasites in 40 (16.0%) participants, while SD Bioline detected 20 (8.0%) positive cases and Akray detected 16 (6.4%) positive cases. Evaluation of the RDTs against microscopy showed that SD Bioline had a sensitivity of 50.0%, specificity of 90.5%, positive predictive value of 50.0%, and negative predictive value of 90.5%. Akray demonstrated a sensitivity of 32.5%, specificity of 87.1%, positive predictive value of 32.5%, and negative predictive value of 87.1%. These findings indicate that SD Bioline performed better than the Akray RDT, although both kits showed lower sensitivity than microscopy.

- *Conclusion:*

The study concludes that microscopy remains the most reliable method for malaria diagnosis among pregnant women. However, SD Bioline rapid diagnostic tests may be used as a useful alternative in settings where microscopy is unavailable, while negative rapid diagnostic tests results in clinically suspected cases should be confirmed by microscopic examination.

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Keywords: *Malaria, Pregnant Women, Microscopy, Rapid Diagnostic Test, Negative Predictive Value, Positive Predictive Value, Akray, SD Bioline.*

I. INTRODUCTION

Malaria remains an important parasitic disease worldwide (WHO 2014). A life-threatening disease caused by *Plasmodium* parasites and transmitted through the bite of female *Anopheles* mosquitoes, remains a significant global health challenge (Fitri *et al.,* 2022). Malaria is prevalent in tropical regions because of the significant amount of rainfall, consistently high temperatures and high humidity, along with stagnant waters in which mosquito larvae readily mature, provide them with the environment they need for continuous breeding. Malaria risk can change throughout the day, over seasons, and across different types of landscapes. Changes in risk of infection are largely driven by the abundance and biting behaviors of malaria vector species (Laurent,2025).

The 6 species of *Plasmodium* that are known to cause disease in humans include *P falciparum*, *P vivax*, *P ovale curtisi*, *P ovale wallikeri*, *P malariae*, and *P knowlesi*. Globally, *Plasmodium falciparum* and *Plasmodium vivax* account for the majority of malaria cases. While *Plasmodium falciparum* is responsible for far more deaths, *Plasmodium vivax* is the most geographically widespread of all of the malaria species, can cause severe, even fatal infections, and resulted in an estimated 9.9 million cases in 2024 (Menkin-Smith 2026).

In 2023, an estimated 263 million cases and 597 000 deaths were reported; with the majority occurring in sub–Saharan Africa (Fitri *et al.,* 2022). It remains a complex and overwhelming health problem, with over 300 to 500 million cases and 2 to 3 million deaths per year. Additionally, pregnant women have two to three times higher risk of suffering from malaria. Due to the loss of maternal immunity and the lack of specialized infection-specific immunity, with typical immunological decline that happens during pregnancy, which is brought on by lack of cell-mediated immune response necessary to maintain the placenta (Robert *et al.,* 2024). This increases their risks of miscarriages, still birth, premature births, low birth weight and anaemia in pregnancy, with children under five years being more susceptible to malaria than adults. All these complications may even lead to death (Okoroiwu 2023). A number of risk factors such as illiteracy, young maternal age, low educational status, joblessness, poor use of insecticide treated net, poverty and inconsistent antenatal care visit have been shown by previous studies to be drivers of malaria infection among pregnant women (Surakat *et al.,* 2023). Nigeria has accounted for 25% and 30% of infant and child deaths respectively and11% maternal mortality (Audu-Ohida *et al.,* 2020). The World Health Organization (WHO) has recommended that all suspected malaria cases must be confirmed with either microscopy for parasitemia counting or RDT in all clinical settings. Since 2010 techniques now are available for malaria diagnosis from the earliest conventional method using microscopy until the novel technique using the rapid molecular method. In the last decade, there has been an interest in the basic immunoassay concept of malaria rapid diagnostic test (Fitri *et al.,* 2022). Various Microscopies has been used for over 100 years and is inexpensive, rapid, and relatively sensitive when used appropriately (Fitri *et al.,* 2022). Rapid diagnostic test is a method that detects malaria antigen in a small amount of blood, usually 5-15µL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip (Wongsrichanalai *et al.,* 2007).

Accurate diagnosis of malaria during pregnancy is essential for ensuring timely treatment, preventing unnecessary use of antimalarial drugs, and reducing adverse maternal and fetal outcomes. Although microscopy remains the reference standard, its application is constrained by limited laboratory facilities, the requirement for trained personnel, and the time needed to obtain results. Rapid diagnostic tests have become increasingly important because they provide quick and convenient diagnosis, particularly in resource-limited settings. However, the performance of RDTs differs among manufacturers, making it necessary to evaluate and compare their diagnostic accuracy before recommending their routine use. This study compares the performance of SD Bioline and Akray RDTs with microscopy to determine their sensitivity, specificity, positive predictive value, and negative predictive value among pregnant women attending Yariman Bakura Specialist Hospital, Gusau. The findings of this study will provide evidence on the relative diagnostic performance of the two RDTs, enabling healthcare providers and hospital management to select the most reliable RDT for routine malaria diagnosis in pregnancy. In addition, the study will contribute to existing knowledge on malaria diagnosis and serve as a useful reference for future researchers and policymakers involved in malaria control programs in Nigeria.

II. MATERIALS AND METHODS

- *Study Design, Population and Area*

Cross-sectional comparative study: comparing malaria diagnosis between two rapid diagnostic tests (SD bioline and Akray) among pregnant women using microscopy as gold standard was conducted. All consenting Pregnant women aged 20-50years with symptoms suggestive of malaria presenting to the hospital and referred to the laboratory for malaria parasite examination were included for the study. After verbal informed consent of the subjects at the point of data collection were considered eligible were enrollment into the study.

- *Sample Size Estimation, Sampling Technique and Data Collection*

The sample size was determined using Yamane's Formula (1967). n = 1+N (e)? The sample size was statistically estimated at 250. Eligible participants were selected by a simple convenient sampling technique. The

methods of data collection comprised personal interview and physical assessment. Semi-structured interviewer-administered questionnaire was used to obtain information on the research variables. The instrument used in the study was structured self-administered questionnaire, which was developed by the researcher based on the objectives of the study.

➢ *Blood Sample Collection*

Five milliliters (5 ml) of blood was aseptically collected from the patient by venipuncture into the plain EDTA container (Audu-ohida *et al.*, 2020).

- *Preparation and Staining of Thick Blood Film:*

A drop of blood was placed on a clean glass slide. The corner of another slide was used to mix the drop of blood in a circular motion over an area of about two centimeter in diameter and was allowed to air dry at room temperature. The smear was allowed to air dry and then flooded with 10% of Giemsa solution. The slide was allowed to stain for 10 minutes, rinsed with clean water and allowed to air dry and was examined by light microscope using oil immersion objective (×100) to confirm the presence of malaria parasites (Bolaji et al., 2023).

- *Procedure for Rapid Diagnostic Test (RDT):*

A drop of blood from the patient was placed on the RDT kit and four 4 drops of buffer was added to form a blood buffer mixture. After 15 minutes, positive result indicates the presence of two bands (test line and control line). For negative results, only the control line appeared. Results were observed and recorded.

- *Determination of Sensitivity, Specificity, Predictive Positive Value and Negative Predictive Value:*

The sensitivity, specificity, predictive positive value and negative predictive value were determined using the following formula:

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

$$Specificity = \frac{TN}{TN + FP} X\ 100$$

$$\text{predictive positive value} = \frac{TP}{TP + FP} \text{ X } 100$$

$$\text{Negative predictive value} = \frac{TN}{TN + FN} X\ 100$$

Where:

TP = True positive
TN = True negative
FP = False positive
FN = False negative
(Deeks, *et al.*, 2023)

➢ *Data Analysis*

The data collected were analyzed using IBM SPSS version 22 computer statistical software package. Quantitative variables were summarized using mean and standard deviation, while qualitative variables were summarized using frequencies and percentages.

➢ *Ethical consideration*

Ethical clearance was obtained from the ethical review committee of Yariman Bakura Specialist Hospital Gusau.

III. RESULTS

Table 1 Prevalence of Malaria Parasites by Microscopy and RDTs Kits Among Subjects (n=250)

Techniques	No of Positive	No of Negative	Prevalence %
Microscopy	40	210	16
SD Bioline	20	230	8
Arkray	13	234	5.2

Total no of pregnant women tested = 250

➢ *Prevalence of Malaria Parasites by Microscopy and RDTs Kits among Subjects (n=250)*

A total of 250 pregnant women were tested for malaria parasite infection using the Rapid Diagnostic Test (RDT) and Microscopic examination. For microscopy, forty (40) of the screened blood samples were positive for malaria while two hundred and ten (210) were negative, yielding a calculated prevalence of 16%. For rapid diagnostic test (SD Bioline), twenty (20) of the screened samples were positive for malaria while two hundred and thirty (230) were negative resulting to a calculated prevalence of 8%. For Akray 13 of the samples examined were positive while 237 were negative resulting to a calculated prevalence of 5.2% (Table 1).

Table 2 Evaluation of RDT Method Using Microscopic Examination as the Gold Standard

Test Kits	SENS	SPEC	PPV%	NPV%
SD Bioline	50	90.5	50	90.5
Arkray	32.5	87.1	32.5	87.1

- *Evaluation of RDT method using microscopic examination as the gold standard*

The Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of SD Bioline were 50%, 90.5%, 50% and 90.5% respectively. While for Akray the Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Values were 32.5%, 87.1%, 32.5% and 87.1 % respectively (Table 2).

IV. DISCUSSION

The findings of this study revealed that out of the 250 pregnant women examined for malaria parasite infection, 40 (16.0%) were positive by microscopy, while 20 (8.0%) and 13 (5.2%) were positive using SD Bioline and Akray rapid diagnostic test (RDT) kits respectively. The higher detection rate observed with microscopy compared with both RDT methods indicates that microscopy remains a more sensitive diagnostic technique for malaria detection, particularly among pregnant women who may present with low parasite densities due to partial immunity or pregnancy-associated changes in immune response. The observed prevalence of 16% demonstrates that malaria remains an important health challenge among pregnant women attending Yariman Bakura Specialist Hospital, Gusau, despite the implementation of malaria control interventions such as insecticide-treated nets, intermittent preventive treatment in pregnancy, and improved access to diagnosis and treatment.

The prevalence recorded in this study is higher than the finding of Bello and Ayede (2019), who reported a prevalence of less than 4% among pregnant women in Ibadan, Oyo State. The lower prevalence reported by Bello and Ayede may be associated with differences in geographical location, environmental conditions, malaria transmission intensity, healthcare accessibility, and preventive practices among the study populations. Ibadan is located in a region with different ecological characteristics and malaria transmission patterns compared with Gusau, where seasonal rainfall, temperature, and mosquito breeding conditions may favour increased malaria transmission.

However, the prevalence observed in the present study is lower than those reported by other researchers. Fana S. et al. (2015) recorded a prevalence of 53.9%, while Bassey et al. (2015) reported 58% among pregnant women in their respective study areas. The higher prevalence reported by these researchers may be attributed to differences in sample population, season of study, malaria endemicity, socioeconomic factors, level of preventive intervention coverage, and diagnostic methods employed. Studies conducted during peak transmission seasons often report higher malaria prevalence due to increased mosquito activity and parasite transmission.

The finding of this study is also comparable with the report of previous researchers who documented moderate malaria prevalence among pregnant women. Differences in malaria prevalence between studies emphasize the influence of local epidemiological factors such as climatic conditions, altitude, availability of healthcare services, community awareness, and utilization of malaria preventive measures. Pregnant women remain highly vulnerable to malaria infection because pregnancy reduces immunity against malaria parasites, increasing the risk of infection and adverse maternal and fetal outcomes.

Microscopy demonstrated a higher ability to detect malaria infection compared with both SD Bioline and Akray RDT kits in this study. This finding agrees with Abbas et al. (2019), who reported that microscopy had higher sensitivity compared with rapid diagnostic tests and remains a reliable method for malaria diagnosis. Microscopy allows direct visualization of malaria parasites in blood films and provides additional information such as parasite species identification, parasite density estimation, and parasite developmental stages. These advantages make microscopy an important reference method for malaria diagnosis, especially in research settings and healthcare facilities with trained laboratory personnel.

The superior performance of microscopy observed in this study is consistent with findings from the World Health Organization (WHO), which recognizes microscopy as the conventional gold standard for malaria diagnosis because of its ability to detect parasites directly and quantify parasitaemia. However, the accuracy of microscopy depends greatly on the experience of the microscopist, quality of reagents, staining procedure, and availability of appropriate laboratory equipment.

The lower detection rates obtained with SD Bioline (8%) and Akray (5.2%) compared with microscopy suggest that both RDT kits missed some malaria-positive cases. This may be due to limitations associated with RDT technology, including reduced ability to detect infections with low parasite density, variation in parasite antigen concentration, degradation of test reagents due to improper storage, and genetic variation affecting parasite antigen expression. Similar observations were reported by previous researchers who noted that RDT sensitivity decreases when parasite density falls below the detection threshold of the test kit.

Comparison of the diagnostic performance of the two RDT kits showed that SD Bioline performed better than Akray, with higher sensitivity, specificity, positive predictive value, and negative predictive value. This finding agrees with studies that have demonstrated variation in performance among commercially available malaria RDTs due to differences in antibody quality, antigen detection capability, and manufacturing standards. The higher sensitivity of SD Bioline suggests that it may be more reliable than Akray for malaria screening among pregnant women in the study area.

The lower sensitivity observed with Akray may be explained by its reduced ability to detect malaria antigens

at low parasite concentrations. Similar findings have been reported by previous studies where some RDT brands showed reduced sensitivity compared with microscopy, particularly in areas with low parasite transmission intensity or among asymptomatic infections. Although RDTs provide advantages such as rapid results, simplicity, and usefulness in areas without microscopy facilities, their limitations must be considered when interpreting negative results.The findings of this study support the observations of previous researchers that while RDTs are valuable tools for malaria diagnosis and have improved access to testing in resource-limited settings, microscopy remains superior in detecting malaria parasites, particularly in vulnerable groups such as pregnant women. Therefore, negative RDT results in pregnant women with clinical suspicion of malaria should be interpreted carefully, and confirmation by microscopy should be encouraged where facilities are available.

Overall, the findings of this study highlight the continued importance of accurate malaria diagnosis among pregnant women. The differences observed between microscopy and RDT results demonstrate the need for continuous evaluation of diagnostic tools before routine implementation. Strengthening laboratory capacity, ensuring quality control of RDT kits, and improving malaria prevention strategies will contribute significantly to reducing the burden of malaria infection among pregnant women in Gusau and other malaria-endemic regions.

V. CONCLUSION

This study reported a prevalence of 16%, 8% and 5.2% using microcopy, SD bioline and Arkray respectively. The finding showed that, the SD Bioline RDT had a sensitivity of 50.0% and specificity of 90.5%, while the Akray RDT had a sensitivity of 32.5% and specificity of 87.1%. Microscopy remains the most reliable method for the diagnosis of malaria in pregnant women. However, RDTs, particularly SD Bioline, can serve as useful alternatives in resource-limited settings where microscopy is unavailable, provided that negative RDT results in clinically suspected cases are confirmed by microscopic examination.

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