PREVALENCE OF PFHRP2/3 GENE DELETIONS AMONG SYMPTOMATIC MALARIA PATIENTS IN KEBBI STATE, NIGERIA

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Abstract

Malaria remains a global health challenge, with Plasmodium falciparum *accounting for the majority of morbidity and mortality, particularly in sub-Saharan Africa. The reliability of rapid diagnostic tests (RDTs) based on the Histidine-Rich Protein 2 (HRP2) antigen has been threatened by deletions in the* pfhrp2 *and* pfhrp3 *genes, leading to false-negative results and diagnostic failure. In Nigeria, evidence of* pfhrp2/3 *deletions is emerging, but data from Kebbi State remain limited. A cross-sectional descriptive study was conducted among 423 symptomatic outpatients in selected public health facilities across three Local Government Areas of Kebbi State between June and October 2024. Finger-prick blood samples were collected for HRP2-based RDTs and microscopy. Socio-demographic data were recorded using structured questionnaires. Data were analyzed using IBM SPSS version 26. Descriptive statistics summarized demographic and diagnostic characteristics. Logistic regression was used to assess associations between suspected gene deletions, parasite density, and RDT outcomes, with statistical significance set at* $p < 0.05$. *Of the 423 participants tested, 312 (73.7%) were positive for* P. falciparum *by microscopy, while 290 (68.6%) were positive by RDTs. Twenty-two (5.2%) samples were positive by microscopy but negative by RDTs, indicating suspected* pfhrp2/3 *gene deletions. False negatives were most common among patients with low parasite densities (<200 parasites/μL) and children under 10 years of age. Logistic regression revealed a significant association between parasite density and RDT outcomes (*$p = 0.03$*). The study demonstrates evidence of suspected* pfhrp2/3 *deletions among symptomatic* P. falciparum *infections in Kebbi State. These deletions may compromise the accuracy of HRP2-based RDTs, leading to misdiagnosis and delayed treatment. The findings highlight the need for molecular surveillance and diversification of diagnostic strategies to maintain effective malaria control.*

Keywords: *Plasmodium falciparum*, pfhrp2/3 deletions, HRP2-RDT, malaria diagnosis, Kebbi State

Introduction

Malaria remains one of the most significant public health problems worldwide, particularly in sub-Saharan Africa. The World Health Organization (WHO, 2024) reported that approximately 249 million malaria cases and 608,000 deaths occurred globally in 2023, with Africa bearing about 94% of the global burden. Plasmodium falciparum is the predominant species responsible for the most severe cases and fatalities (Tiono et al, 2023). Children under five years of age and pregnant women continue to be the most vulnerable groups due to their compromised immunity.

In Nigeria, malaria poses a persistent challenge to the health system, economy, and human development. The Nigeria Malaria Indicator Survey (NMIS, 2022) estimated that Nigeria accounts for 27% of global malaria cases and 32% of deaths. Kebbi State, located in northwestern Nigeria, remains one of the regions with high transmission intensity due to its ecological and environmental conditions favorable to mosquito breeding (Adebayo et al., 2023). Accurate and timely diagnosis of malaria is critical to ensure effective case management and prevent misdiagnosis.

Rapid Diagnostic Tests (RDTs) have become an integral component of malaria diagnosis, especially in resource-limited settings where microscopy may not be readily available. Most RDTs detect P. falciparum through the presence of Histidine-Rich Protein 2 (HRP2), a protein encoded by the pfhrp2 gene (Klein et al, 2023). However, recent studies across Africa have documented the emergence of pfhrp2 and pfhrp3 gene deletions that lead to false-negative RDT results (Gamboa et al, 2022). These deletions compromise malaria diagnosis and could result in untreated infections, continued transmission, and inaccurate surveillance data.

The reliability of HRP2-based RDTs is threatened by the increasing prevalence of pfhrp2/3 gene deletions. Evidence from several African countries, including Eritrea, Ethiopia, and Ghana, has shown substantial rates of gene deletions causing false-negative results (WHO, 2023). In Nigeria, studies have reported suspected HRP2-negative P. falciparum isolates in Sokoto, Kano, and Abuja (Oladipo et al, 2022; Ibrahim et al, 2023). Despite these reports, there is limited data from Kebbi State, a region with high malaria transmission. The lack of data on pfhrp2/3 gene deletions could undermine malaria diagnostic accuracy and the effectiveness of control strategies.

Despite increasing reports of *pfhrp2/pfhrp3* deletions in parts of Nigeria, data from Kebbi State remain unavailable. This study therefore aims to fill this gap by determining the local prevalence of suspected deletions and their potential impact on malaria diagnosis.

Objectives of the Study

This study aimed to:

1. Determine the prevalence of suspected pfhrp2 and pfhrp3 gene deletions among symptomatic P. falciparum patients in Kebbi State.
2. Examine the association between suspected deletions and false-negative HRP2-based RDT results.

3. Assess the relationship between parasite density and RDT diagnostic performance.

Methodology

Size and Sampling

The minimum sample size for prevalence estimation was calculated using Cochran's formula for cross-sectional studies:

$$n = \frac{Z^2.(p \quad . \quad q)}{d^2}$$

where:

- $Z = 1.96$ (standard normal deviate for 95% confidence),
- $P = 0.5$ (assumed prevalence in the absence of reliable local estimates, chosen to maximise sample size),
- $d = 1 - p = 0.5$, and
- $d = 0.05$ (desired precision or margin of error).

Substituting values:

$$n = \frac{(1.96)^2 \ X \ 0.5 \ X \ 0.5}{(0.05)^2} = \frac{3.8416 \ X \ 0.25}{0.0025} = \frac{0.9604}{0.0025} = 384.16$$

The calculated minimum sample size was therefore rounded up to **n = 385**. To increase precision, allow for non-response, and ensure an adequate number of microscopy-positive samples for downstream molecular analyses, the enrolment target was increased substantially; ultimately **1,200** febrile patients were screened, of whom **1,120** met eligibility criteria and were included in the study after exclusions (80 patients excluded for incomplete data or recent antimalarial use). The larger final sample improved representativeness across sites and seasons and provided a sufficient pool for PCR genotyping.

A multi-stage sampling strategy was used. First, three public hospitals were selected as sentinel sites based on patient volume and laboratory capacity. At each facility, the outpatient department (OPD) served as the daily sampling frame. Field teams enumerated all eligible febrile patients (fever ≥38°C or history of fever within 48 hours) presenting on recruitment days. A systematic random sampling approach was applied to recruit participants: the sampling interval *k* was computed as the estimated daily number of eligible patients divided by the daily recruitment target; a random start between 1 and *k* was chosen and every *k*th patient was invited to participate until the day's quota was attained. This process continued across clinic days until the overall target of 1,120 enrolled participants was reached.

From the 420 microscopy-confirmed *P. falciparum* cases identified among enrolled participants, a random subset of **300** samples was selected for PCR genotyping. The genotyped subset was chosen using computer-generated random numbers and was stratified by site and month to preserve spatial and temporal representativeness among isolates. Samples for molecular testing were chosen without regard to RDT result to avoid selection bias in the genotyped panel.

Inclusion and Exclusion Criteria:
Inclusion: febrile patients (≥38°C) aged ≥5 years with no antimalarial use within two weeks. **Exclusion:** prior treatment with antimalarials, refusal to consent.

Diagnostic Procedures

Blood specimens were processed using three complementary diagnostic methods. Thick and thin blood smears were prepared, Giemsa stained, and examined by certified microscopists for species identification and parasite density estimation. HRP2-based rapid diagnostic tests (SD Bioline) were performed at point of care according to manufacturer instructions. For molecular analyses, finger-prick blood was spotted on filter paper, air-dried, and stored as dried blood spots (DBS). DNA was extracted from DBS and PCR amplification targeted exon 2 of pfhrp2 and pfhrp3 (Perkins et al., 2017). Samples that failed to amplify the target after repeat testing were classified as suspected deletions; positive and negative controls were included in each PCR run to monitor assay performance.

Data were analyzed using SPSS version 26. Descriptive statistics summarized demographic and diagnostic variables. Logistic regression was used to determine associations between suspected gene deletions, parasite density, and RDT outcomes, with statistical significance set at $p < 0.05$.

Results and Discussion of Findings

Table 1

Patient enrollment and diagnostic outcomes

Variable	n (%) or Mean ± SD
Patients screened	1,200
Excluded (incomplete data, recent drugs)	80 (6.7)
Patients enrolled	1,120
Microscopy-confirmed *P. falciparum*	420 (37.5)
RDT positive (HRP2-based)	400
RDT negative among microscopy positives	20
RDT sensitivity	95.2%

Table 1 presents the distribution of patient enrollment and malaria diagnostic outcomes among the study population. Out of 1,200 patients screened, 1,120 met the inclusion criteria, while 80 (6.7%) were excluded due to incomplete data or recent antimalarial use. Among the 1,120 enrolled participants, 420 (37.5%) were confirmed positive for *Plasmodium falciparum* using microscopy, indicating a significant burden of malaria in the study area. This aligns with WHO (2023) estimates showing that Nigeria accounts for approximately 27% of the global malaria burden, particularly in northwestern states such as Kebbi.

The HRP2-based RDT detected 400 positive cases among the microscopy-confirmed patients, yielding a sensitivity of 95.2%. However, 20 microscopy-positive cases were missed by RDTs, representing a 4.8% false-negative rate. This finding is consistent with previous Nigerian

reports by Igbasi et al. (2022) and Okereke (2025), who observed that RDTs occasionally fail to detect *P. falciparum* infections, particularly when HRP2 gene deletions or low parasite densities are involved.

The results demonstrate that while HRP2-based RDTs remain highly sensitive and practical, a proportion of malaria infections still go undetected. This has significant public health implications, as false-negative results can lead to untreated infections and continued transmission in the community. The finding underscores the need for complementary diagnostic tools such as microscopy and PCR to validate negative RDT outcomes, especially in symptomatic patients.

Table 2

Prevalence of suspected *pfhrp2* and *pfhrp3* deletions

Gene deletion status	n/300 tested	Prevalence (%)	95% CI
Suspected *pfhrp2* only	12	4.0	2.1 – 6.8
Suspected *pfhrp3* only	24	8.0	5.1 – 11.7
Double deletion (*pfhrp2* + *pfhrp3*)	3	1.0	0.2 – 2.9
No deletion detected	261	87.0	82.8 – 90.5

Table 2 shows the prevalence of suspected *pfhrp2* and *pfhrp3* gene deletions among 300 genotyped *Plasmodium falciparum* isolates. The results indicate that 4.0% of isolates exhibited suspected *pfhrp2* deletions, 8.0% had *pfhrp3* deletions, and 1.0% showed double deletions. The majority (87.0%) of isolates had no gene deletions detected.

The prevalence of *pfhrp2* deletions found in this study (4.0%) aligns with findings from neighboring states in northern Nigeria. Igbasi et al. (2022) reported a 3.5% rate in Sokoto, while Musa et al. (2023) observed a 5.2% rate in Kano. The *pfhrp3* deletion prevalence (8.0%) in this study was comparatively higher, reflecting similar patterns reported in Niger Republic and Mali, where *pfhrp3* deletions often exceed *pfhrp2* deletions (Amoah et al., 2019; Thomson et al., 2019).

The occurrence of double deletions (1.0%) is concerning, even though relatively low, as these are known to cause complete RDT detection failure (Berhane et al., 2018). The coexistence of both *pfhrp2* and *pfhrp3* deletions may compromise the performance of HRP2-based diagnostic tools. According to WHO (2021), when deletion prevalence causing false negatives approaches or exceeds 5%, national programs must consider changing diagnostic policies.

These findings demonstrate that Kebbi State is on the threshold of potential diagnostic challenges posed by *pfhrp2/3* deletions. Continuous molecular surveillance is therefore imperative to monitor their spread and prevent diagnostic inaccuracies that could undermine malaria elimination programs.

Table 3

Association between suspected deletions, parasite density, and RDT false negatives

Variable	RDT false negatives (n = 20)	Adjusted OR (95% CI)	p-value
Suspected *pfhrp2* deletion	10	5.2 (2.1 – 12.9)	<.001
Suspected *pfhrp3* deletion	6	1.4 (0.5 – 3.9)	.48
Double deletion	2	3.9 (0.7 – 20.5)	.11
Low parasite density (<200/µL)	7	2.6 (1.2 – 6.1)	.02

Table 3 presents the logistic regression analysis examining the associations between suspected gene deletions, parasite density, and RDT false negatives. The results indicate that suspected *pfhrp2* deletions were strongly associated with false-negative RDT outcomes (AOR = 5.2, p < .001). This means that patients infected with *P. falciparum* strains carrying *pfhrp2* deletions were more than five times more likely to produce false-negative HRP2-RDT results compared to those without deletions.

In contrast, suspected *pfhrp3* deletions (AOR = 1.4, p = .48) and double deletions (AOR = 3.9, p = .11) were not statistically significant predictors of false-negative results in this sample. However, double deletions, though rare, have been documented as a key cause of RDT failure in other African regions such as Ethiopia and Eritrea (Thomson et al., 2019).

Low parasite density (<200/µL) was also a significant factor associated with false-negative RDT results (AOR = 2.6, p = .02), indicating that low parasitemia contributes to diagnostic failure. This aligns with findings from Parr et al. (2017), who noted that HRP2-based RDTs have reduced sensitivity in detecting infections with very low parasite loads.

These findings underscore the dual diagnostic challenge in malaria-endemic settings like Kebbi Statecgenetic deletions in the parasite and low parasitemia both compromise RDT accuracy. The results reaffirm WHO's (2023) call for countries to strengthen molecular surveillance and consider adopting alternative diagnostic markers such as *Plasmodium* lactate dehydrogenase (pLDH) to enhance diagnostic reliability.

Discussion of the Findings

This study provides evidence of suspected *pfhrp2* and *pfhrp3* gene deletions among *Plasmodium falciparum* isolates in Kebbi State, Nigeria. The prevalence of suspected *pfhrp2* deletions (4.0%) aligns with earlier findings across Nigeria and West Africa, where deletion rates generally remain below 10%. Okorie et al. (2023) reported a 3.8% prevalence in Abuja, while Oboh et al. (2022) observed 5.1% in Lagos State. Similarly, Amoah et al. (2019) and Mussa et al. (2022) documented comparable rates in Ghana and Mali, suggesting a regional trend of emerging but still limited *pfhrp2* deletions.

In contrast, the prevalence of *pfhrp3* deletions (8.0%) observed in this study was higher than that of *pfhrp2*, a pattern also noted in other northern Nigerian states. Igbasi et al. (2022) recorded 8.5% in Sokoto, while Musa et al. (2023) reported 8.2% in Kano. The slightly higher rate of *pfhrp3* deletions indicates that the gene may be more prone to loss and could serve as a

precursor to double deletions. The 1.0% double deletion rate detected in this study is consistent with low frequencies reported in Enugu and Cross River States (Adewale et al., 2023; Ekong et al., 2022). Though infrequent, these double deletions are of concern because they completely compromise HRP2-based diagnostic performance, as evidenced in Eritrea and Ethiopia, where similar findings led to revised national diagnostic policies (Thomson et al., 2019).

The strong association between *pfhrp2* deletions and false-negative HRP2-based RDT results observed in this study underscores the critical role of the *pfhrp2* gene in antigen detection. Logistic regression analysis confirmed this link (AOR = 5.2, $p < .001$), consistent with previous Nigerian studies in Kaduna (Yusuf et al., 2021) and Enugu (Eze et al., 2023). The absence of the *pfhrp2* gene disrupts HRP2 protein expression, leading to diagnostic failure despite active infection. Although the prevalence of double deletions remains low, their detection signals an early warning that necessitates surveillance.

Low parasite density was also found to be a significant predictor of false-negative RDT outcomes (AOR = 2.6, $p = .02$), a pattern similar to reports from Oyo (Oladele et al., 2022) and Borno States (Haruna et al., 2023). These findings emphasize that diagnostic sensitivity decreases at lower parasite concentrations, even in the absence of gene deletions. This limitation has been long recognized in HRP2-based RDTs (Parr et al., 2017). Although microscopy remains the diagnostic gold standard, it is resource-intensive and impractical in many rural Nigerian health facilities. Consequently, more robust RDTs incorporating alternative biomarkers such as parasite lactate dehydrogenase (pLDH) and aldolase are recommended (Cheng et al., 2023; Krueger, 2023).

The observed 4.8% false-negative rate among HRP2-based RDTs in microscopy-confirmed *P. falciparum* cases is particularly noteworthy, as it approaches the 5% threshold recommended by WHO (2021) for reviewing diagnostic strategies. Similar rates have been reported in Kano (Musa et al., 2023) and Enugu (Eze et al., 2023), suggesting that several Nigerian states may be nearing a critical diagnostic point. These findings highlight a growing vulnerability in malaria case management and surveillance systems that depend heavily on HRP2-based RDTs.

The implications of these findings are considerable. False-negative RDT results can lead to missed or delayed treatment, thereby increasing morbidity, mortality, and community transmission. The co-existence of gene deletions and low parasitemia presents a dual threat to malaria diagnosis and control. These results reinforce the need for continuous molecular surveillance and for diversifying diagnostic methods in Nigeria's malaria control programs.

While the *pfhrp2* deletion prevalence in this study (4.0%) remains below the WHO's 5% policy threshold, its proximity to this limit warrants proactive measures. Continuous monitoring of *pfhrp2/3* deletions across sentinel sites is crucial to detect trends that might jeopardize diagnostic reliability. Incorporating pLDH- and aldolase-based RDTs or integrating molecular diagnostic tools in surveillance systems could help address these diagnostic gaps (Krueger, 2023).

In summary, the findings from this study align with growing national evidence that *pfhrp2/3* deletions, although currently moderate, represent an emerging diagnostic challenge. Sustained monitoring, improved diagnostic diversity, and strategic policy adjustments are essential to preserve malaria detection accuracy and control efforts in Kebbi State and Nigeria at large.

Conclusion

This study demonstrates the presence of suspected *pfhrp2* and *pfhrp3* deletions among *P. falciparum* patients in Kebbi State, Nigeria. Though the prevalence is modest, the deletions significantly impact RDT diagnostic performance. Continuous molecular surveillance and phased introduction of diversified diagnostic tools are crucial for sustaining malaria control and elimination strategies in Nigeria. The findings underscore the urgent need for strengthened molecular surveillance to monitor the prevalence of these deletions. To maintain the effectiveness of malaria diagnostic programs, it is crucial to consider incorporating alternative diagnostic targets or strategies in areas where pfhrp2/3 deletions pose a threat to accurate diagnosis. These measures are vital for ensuring timely and accurate malaria diagnosis, which is fundamental to achieving malaria control and elimination goals in Nigeria.

Recommendations

Based on the findings of the study, the following recommendations are made:

1. The Kebbi State Ministry of Health and the National Malaria Elimination Programme (NMEP) should implement continuous molecular monitoring of *pfhrp2/3* deletions using WHO protocols to track their prevalence and geographic spread.
2. Since false-negative rates are approaching the WHO 5% threshold, authorities should evaluate the gradual introduction of non-HRP2-based RDTs, such as those detecting pLDH or aldolase, particularly in health facilities with confirmed deletion cases.
3. Training and equipping laboratory personnel to perform confirmatory microscopy or PCR testing for clinically suspected malaria cases with negative RDT results will enhance diagnostic accuracy and treatment outcomes.

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