

Prevalence of *Plasmodium falciparum* Infection and Assessment of Histidine-Rich Protein (Pfhrp2 and Pfhrp3) Following Antimalarial Treatment Among Lactating Mothers and Infants in Dutsin-Ma Local Government Area, Katsina State

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ABSTRACT

Malaria Rapid Diagnostic Test antigen persistence complicates treatment monitoring in endemic areas. This longitudinal therapeutic efficacy study assessed HRP2/HRP3 persistent positivity after ACT and Fansidar treatment and identified associated biofactors among 360 participants 180 infants 0-2 years and 180 lactating mothers 19-40 years with microscopy-confirmed *Plasmodium falciparum* at General Hospital Dutsin-ma. Participants received Artemether-lumefantrine ACT, N=119 (66.1%) or Sulfadoxine-pyrimethamine Fansidar n=61 (33.9%). Follow-up was on Day 1-4, 5-9, 10-14, 15-19 and 20-24. HRP2/HRP3 clearance was assessed by RDT. Biofactors PfCRT K76T, anemia, HIV were analyzed using Chi-square and pearson correlation at p<0.05. At baseline Day 1-4, 100% were HRP2 positive and 91.1% HRP3 positive. Persistence declined progressively; Day 5-9, 99.4% HRP2; Day 10-14, 46.7% infants and 47.2% mothers; Day 15-19, 3.9%; Day 20-24, 0% clearance in both groups. Age significantly influenced clearance p<0.001: infants 0-0.3 years 25.0% vs >2.0 years 83.3% on Day 10-14; mothers 19-21 years 25.8% vs >40 years 85.7%. ACT group had 63.0% persistence vs 18.0% Fansidar on Day 10-14 p<0.001. PfCRT showed strongest correlation with persistence r=0.65, followed by anemia r=0.50 and HIV r=0.45. Despite eventual clearance by Day 20-24, delayed clearance in ACT group is concerning and suggests reduced efficacy or tolerance. Persistence is multifactorial driven by parasite resistance marker and host factors.

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INTRODUCTION

Malaria is a life-threatening disease caused by parasites of the genus *plasmodium* and transmitted to humans through the bite of infected female Anopheles mosquitoes (Snow, 2017). *Plasmodium falciparum* is the most prevalent and deadly species in sub-Saharan Africa, accounting for over 90% of malaria cases and deaths in Nigeria (WHO, 2023). Nigeria bears the highest burden of malaria cases and 31% of deaths (WHO, 2023). Katsina State in Northwest Nigeria is a high transmission area with perennial malaria transmission. Peaking during the rainy season from May to October (FMOH, 2020). The current first-line treatment for uncomplicated *P. falciparum* malaria in Nigeria is Artemisinin-based Combination Therapy (ACT) (FMOH, 2020). ACTs have been effective in reducing malaria morbidity and mortality. However, the emergence of drug resistant *P. falciparum* is a major threat to malaria control and elimination efforts (WHO, 2022). Therapeutic Efficacy Studies (TES) are the gold standard for monitoring antimalarial drug efficacy. World Health Organization recommends that a treatment failure rate of >10% after PCR correction or delayed parasite clearance on Day 3 should trigger a change in treatment policy (WHO, 2009). Recent reports from East Africa have shown emergence of Kelch 13 mutations associated with artemisinin resistance and delayed parasites clearance (WHO, 2022b). Lactating mothers and infants are a vulnerable group. During lactations, physiological changes affect drug absorption, distribution and excretion (WHO, 2015). Infants have immature immunity and anemia, despite this, most TES and drug resistance

surveillance in Nigeria focus on children under 5 years and general adults. Data specific to lactating mother's infants remain limited. The global spread of drug resistance is greatly facilitated by modern air travel. Millions of people become colonised (usually without symptoms) with resistant pathogens or other (non-pathogenic) microbes containing genetic determinants of resistance every year in locations with high rates of resistance and then fly to regions where (whether or not those colonised are sick) resistant pathogens and/or resistance determinants are directly or indirectly transmitted to others (Kennedy and Collignon, 2010). The direct selection of resistance following exposure of children carrying pneumococci has been shown in a prospective study in Malawi to occur in 42 percent of children exposed to sulfadoxine-pyrimethamine for a week and in 38 percent of children a month after exposure to drug treatment for malaria (Feikin *et al.*, 2000).

Chloroquine-resistant strains of *Plasmodium falciparum* malaria appeared a half-century ago in Southeast Asia and South America and spread across Africa, especially East Africa, in the past quarter-century (Wellems & Plowe, 2001). Patients infected with resistant strains are more likely to be sicker, to be hospitalized for longer periods of time, and to die of the infection (Carmeli *et al.*, 2002). Malaria parasites display a multifaceted life cycle relating sporadic cycles of asexual division (Schizogony) occurring in man: an intermediary host and sexual development (sporogony) occurring in female anopheles' mosquito which serves as an ultimate host (Olawe, *et al.*, 2015). An ancient illness called malaria is derived from the Italian (mal-aria), which means

"foul air." It was also known by the names Roman fever, ague, marsh fever, periodic fever, and palladium (Olowe, *et al.*, 2007). The prevalence of *pfmdr1* polymorphisms in Nigeria was reported from the Southern region, where a positive association between *pfmdr1* N86, F184 and D1246 alleles and medical failure was observed (Happi CT, *et al.*, 2020). In dissimilarity, a prevalence of 62.2% and 69.0% for *pfmdr1* 86Y and F184 allele, respectively, was also documented from the region (Oladipo *et al.*, 2015) which was recently followed by another survey where the *pfmdr1* 86Y and 1246Y alleles had prevalence of 24% and 18.6%, respectively. (Dokunmu *et al.*, 2019). Yet there is no valid baseline data involving *pfmdr1* SNPs in both North-West and North-East Nigeria since the withdrawal of CQ and adoption of AL in Northern Nigeria. Malaria is a severe febrile illness in humans which can sometimes be fatal especially in young children (Riley *et al.*, 2020). Malaria remains a leading cause of morbidity and mortality among lactating mothers and infants in Nigeria, particularly in regions such as Dutsin-Ma Katsina State. Despite the availability of antimalarial treatments, the emergence poses a significant threat to effective disease management. Current literature indicates a rising trend in resistance patterns however. The aim of this research is to investigate the prevalence of *Plasmodium falciparum* infection and assessment of Histidine rich protein (*pfhrp2* and *pfhrp3*) following antimalarial treatment among lactating mothers and infants in Dutsin-ma local government Katsina State. The objectives of the study are; To determine the prevalence of *P. falciparum* among lactating mothers and infants, To determine the Histidine Rich Protein (*pfhrp2/pfhrp3*) persistent positivity pattern after antimalarial treatment among lactating mothers and their infants, To compared the Persistent Positivity of Antigen of RDTs used after Antimalarial Drug Administration (ACT and Fansider), To identified associated Biofactors with Persistent Positivity of Antigen of RDTs used after Antimalarial Drug Administration and Correlation between Biofactors and Persistence Positivity of RDT Antigen.

Research Hypotheses

H1:

H₀= There is no significant prevalence of *P. falciparum* among lactating mothers and infants.

H₁= There is significant prevalence of *P. falciparum* among lactating mothers and infants.

H2:

H₀= There is no significant difference of Histidine Rich Protein (*pfhrp2/pfhrp3*) persistent positivity pattern after antimalarial treatment among lactating mothers and their infants.

H₁= There is significant difference of Histidine Rich Protein (*pfhrp2/pfhrp3*) persistent positivity pattern after antimalarial treatment among lactating mothers and their infants.

H3:

H₀= There is no significant Persistent Positivity of Antigen of RDTs used after Antimalarial Drug Administration (ACT and Fansider).

H₁= There is significant Persistent Positivity of Antigen of RDTs used after Antimalarial Drug Administration (ACT and Fansider).

H4:

H₀= There is no significant association between biofactors PFCRT, anemia, HIV and persistent positivity of RDT antigen.

H₁= There is significant association between biofactors PFCRT, anemia, HIV and persistent positivity of RDT antigen.

MATERIALS AND METHODS

Study Area

Dutsinma is located on latitude (12°27'6.012" E) and longitude (7°29'52.98" N). The inhabitants are mostly Hausa, Fulani speaking people constitute a population of 169,671 according to census 2006; the area is characterized by mean temperature ranging between 35°C - 39°C annually. The vegetation is dominated by trees, shrubs and grasses. Majority of the indigenes are farmers, civil servants, fishermen and others are teachers and businessmen; General Hospital Dutsinma will be used for pilot testing.

Study Population

The study population comprised Lactating Mothers and Infants who's attended Dutsinma General Hospital between May to October 2023 were recruited and the study has the inclusion and exclusion criteria during study period. The study population was categorized into two groups for sample size allocation: group A: Lactating Mothers aged 19 – 49 years who's actively breastfeeding an infant and group B: Infants aged 0 – 24 months, biological of the lactating mothers recruited. A total of 360 participants were recruited, consisting of 180 Lactating mothers and 180 Infants.

Inclusion and Exclusion Criteria

Inclusion criteria: Lactating mothers aged 19 to 49 years currently breastfeeding an infant, Infants aged 0 to 24 months and resident of Dutsinma Local Government for at least 6 months who gave informed consent and Infants with confirmed *P. falciparum* mono-infection and history of fever in last 24hours for TES component. Exclusion criteria: Mothers with severe illness or unable to give consent, infants with severe malaria, danger signs or other co-morbidities. Infants who received antimalarial in the past 2 weeks. Mother – infant pairs planning to relocate outside Dutsinma within 28 days.

Sample Size Determination

The sample size was calculated using cochrane's formula for estimating a single proportion:

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

Where:

Z = 1.96 at 95% Confidence interval

P = 0.5 expected prevalence. 50% was used because there is no previous study in the Area

d = 0.054, desired precision / margin of error.

Therefore, n = 327

Adjusting for a 10% non-response rate: 327 × 1.1 = 360

Sample Collection

Samples were collected from participants drawn from out-patients visiting selected hospital in Dutsinma Local Government Area with their consent.

Blood Collection: Blood sample was collected using a 5ml syringe through the venous vein. The site was cleaned using 70% alcohol; and allowed to dry before blood sample collection. Blood sample collected were transferred into EDTA vacuum tube, containing anticoagulant and clearly labeled, indicating: date, time of collection as well as serial number and location of patient or volunteer (CDC, 2016).

Preparation of Thick and Thin Film: Thick and thin smears were prepared using a pre cleaned slides (frosted – end) and labeled with date, time of collection as well as serial number and location of patient or volunteer (CDC, 2016).

Preparation of Thick Film: Thick blood film was prepared for malaria parasite detection and quantification according to WHO (2016) guidelines. The glass slide was labeled with the participant's ID number, date, and 'T' for thick film. 3-4 drops of blood were placed close together in the center of the slide to form an area of about 1-1.5cm in diameter. Using the applicator stick, the blood drops were gently mixed and spread in a circular motion to form a uniform thick film. The thick film was air-dried horizontally in a flat position for 1-2 hours.

Preparation of Thin Film: Thin blood film was prepared for species identification and parasite quantification according to WHO (2016). The glass slide was labeled with the participant's ID number, at one end. A small drop of blood, approximately 2-3mm in diameter was placed about 1cm from the frosted end of the slide. A clean spreader slide held at a 45° angle was placed in front of the blood drop and allowed to spread along the edge by capillary action. The spreader was then pushed forward smoothly and steadily to produce a thin film of blood. The thin film was air-dried horizontally for 10-15 minutes. The dried thin film was fixed by dipping briefly in 100% methanol for 3 seconds to fix the red blood cells. The slide was then air-dried again.

Microscopic Examination

The prepared slides were examined using the 100 × oil immersion objective lens and reported as follows: Positive (presence of parasite): at least 300 fields were considered as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) or Clinical and Laboratory Standards Institute (CLSI) (2005). Negative "No Parasites Found" (NPF), is to be consider at least 100 fields, each containing approximately 20 WBCs be screened according to WHO recommendation (CDC, 2016).

Follow-Up Using Rapid Diagnostic Tests for Assessment of *Plasmodium Falciparum* Drug Resistance

Follow-up was conducted according to modified WHO 28days TES protocol. Participants were scheduled for follow-up visits on Day 1-4, Day 5-9, Day 10-14, Day 15-19, Day 20-24 post-treatment. At each follow-up visit: Clinical assessment for fever, vomiting, and other symptoms was done, Finger-prick blood sample was collected. HRP2/HRP3 Rapid Diagnostic. Test was performed to detect *P. falciparum* antigen.

Thick and thin blood films were prepared for microcopy confirmation.

Data Analysis

Data were entered and analyzed using Statistical Package for Social-Sciences (SPSS) version 21.0. Descriptive statistics was used to determine frequencies and percentages. Chi-square test and Independent T-test were used to compare categorical variables between groups. Pearson correlation was used to determine correlation between biofactors and persistent positivity of RDT Antigen. Statistical significance was set at $P < 0.05$.

Rapid Diagnostic Tests (RDTs)

Rapid diagnostic tests (RDTs) kits are used to determine whether someone exhibiting malaria-like symptoms truly has the disease. The parasite that causes malaria infects blood cells (Oduola. *et al*, 2021). Antigen in *plasmodium falciparum* was detected using RDT kit Manufactured by Premier Medical Corporation Private Limited A1-302, GIDC, Sarigam 396155.Dist. Valsad, Gujarat, INDIA www.premiermedcorp.com. Mfg. Lic. No. MFG/MG/2018/000064. This kit identifies particular antigens (proteins) made by malaria parasites. Those who are frequently infected or who have just contracted the disease have these antigens in their blood. An absorbent nitrocellulose strip changes color when antigen is present, signaling the presence of the substance. One species (*Plasmodium falciparum*) can only be detected by some Rapid diagnostic tests (RDTs), which typically do so by looking for Histidine-rich protein2 (HRP2) or parasite-specific lactate dehydrogenase (PLDH) (WHO 2020).

Ethical Approval

Ethical approval was required from the research ethic review committees of Katsina State Ministry of Health through Katsina State Hospital Management Board.

RESULTS AND DISCUSSION

360 samples in total were evaluated, 180 lactating mothers and 180 infants.

Overall prevalence was 59.2%. Prevalence was higher in lactating mothers 68.9% than infants 49.4%, Chi-square=14.12, $p=0.001^*$ significant.

Table 1: Overall Prevalence of *Plasmodium Falciparum* Among Lactating Mothers and Infants

Study Group	Number Examined	Number Positive %	Number Negative %
Lactating Mothers	180	124 (68.9%)	56 (32.1%)
Infants 0-24 months	180	89 (49.4%)	91 (50.6%)
Total	360	213 (59.2)	147 (40.8%)

As shown in table 4.1, all 180 (100%) infants were HRP2 Positive at baseline. There was a progressive decline in antigen positivity with time. By Day 10-14, only 84 (46.7%)

were HRP2 positive and 58 (32.2%) were HRP3 positive. Complete clearance was achieved by Day 20-24. Age was significantly associated with clearance rate $p<0.01$.

Table 2: Histidine Rich Protein2 and Histidine Rich Protein3 Persistence Positivity After Antimalarial Treatment Among Infants Attending General Hospital Dutsin-Ma Katsina State

Age Groups (Years)	Total Number Examined	Post Intervention (Day 1 to 4)		Post Intervention (Day 5 to 9)		Post Intervention (Day 10 to 14)		Post Intervention (Day 15 to 19)		Post Intervention (Day 20 to 24)	
		HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)
0 to 0.3	60	60 (100.)	58 (97.0)	59 (98.3)	58(96.7)	15 (25.0)	5 (8.3)	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)
0.3 to 0.6	36	36 (100.)	34 (94.4)	36 (100.0)	34 (94.4)	13 (36.1)	10 (27.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0.6 to 0.9	21	21 (100.)	19 (90.5)	21 (100.0)	19 (90.5)	11 (52.4)	10 (47.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1 to 1.5	28	28 (100.)	23 (82.1)	28 (100.0)	23 (82.1)	18 (64.3)	11 (39.3)	3 (10.7)	1 (3.6)	0 (0.0)	0 (0.0)
1.5 to 2	23	23 (100.)	21 (91.3)	23 (100.0)	21 (91.3)	17 (73.9)	15 (65.2)	2 (8.7)	0 (0.0)	0 (0.0)	0 (0.0)
> 2.0	12	12 (100.)	9 (75.0)	12 (100.0)	9 (75.0)	10 (83.3)	7 (58.3)	1 (8.3)	1 (8.3)	0 (0.0)	0 (0.0)
Total	180	180 (100.)	164 (91.1)	179 (99.4)	164 (91.1)	84 (46.7)	58 (32.2)	7 (3.9)	2 (1.1)	0 (0.0)	0 (0.0)
Chi-square		NA	9.843	1.936	9.843	37.491	33.685	5.214	4.102	NA	NA
Df		NA	5	5	5	5	5	5	5	NA	NA
p Value		NA	0.080ns	0.858ns	0.080ns	<0.001*	<0.001*	0.389ns	0.536ns	NA	NA

Keywords; HRP=Histidine Rich Protein, Df=Significance different, NA=Not average, ns=not significance, <=less significance

Table 4.2 shows similar pattern among lactating mothers. Baseline positivity was 100% for HRP2. Significant age related difference was observed with mothers >40 years showing delayed clearance 84.6% on Day 10-14 compared to 25.4% in 19-21 years, p-0.006. Complete clearance occurred by Day 20-24.

Table. 2.1: Histidine Rich Protein2 and Histidine Rich Protein3 Antigen Clearance After Antimalarial Treatment Among Lactating Mothers Attending General Hospital Dutsin-Ma Katsina State

Age Groups (Years)	Total Number Examined	Post Intervention (Day 1 to 4)		Post Intervention (Day 5 to 9)		Post Intervention (Day 10 to 14)		Post Intervention (Day 15 to 19)		Post Intervention (Day 20 to 24)	
		HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)
19 to 21	59	59(100)	57(97.0)	98.5	97	25.8	9.1	1.5	0	0	0
22 to 24	36	36(100)	34(95)	100	95	37.5	27.5	0	0	0	0
25 to 27	21	21(100)	19(91)	100	91.3	52.2	47.8	0	0	0	0
28 to 30	28	28(100)	23(82.1)	100	80.6	64.5	38.7	9.7	3.2	0	0
31 to above	23	23(100)	21(91.3)	100	92	76	64	8	0	0	0
> 40	13	13(100)	10(76.9)	100	78.6	85.7	64.3	7.1	7.1	0	0
Total	180	180(100)	164(91.1)	99.5	91.5	47.7	32.7	3.5	1	0	0
T-test		3.493		3.402		1.173		1.241		NA	
Df		10		10		10		10		NA	
p Value		0.006*		0.007*		0.268ns		0.243ns		NA	

Keywords; HRP=Histidine Rich Protein, Df=Significance different, NA=Not average, ns=not significance, <=less significance.

The table 3. shows that 119(66.1%) received ACT while 61(33.9%) received Fansider. Participants treated with ACT had significantly higher persistent antigenemia on Day 10-14: 63.0% HRP2 and 46.2% HRP3 compared to 18.0% and 6.6% in Fansider group respectively, p<0.001. Both groups achieved 0% positivity by Day 20-24.

Table 3: Persistent Positivity of Antigen of RDTs used after Antimalarial Drug Administration (ACT and Fansider)

Intervention (Malaria Treatment)	Total Number Examined	Post Intervention (Day 1 to 4)		Post Intervention (Day 5 to 9)		Post Intervention (Day 10 to 14)		Post Intervention (Day 15 to 19)		Post Intervention (Day 20 to 24)	
		HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)
ACT	119	119 (100.0)	108(90.8)	119 (100.0)	108 (90.8)	75 (63.0)	55 (46.6)	5 (4.2)	1 (0.8)	0 (0.0)	0 (0.0)
Non-ACT	61	61 (100.0)	56 (91.8)	60 (98.5)	56 (91.8)	11 (18.0)	4 (6.6)	1 (1.6)	1 (1.6)	0 (0.0)	0 (0.0)
Total	180	180 (100.0)	164 (91.1)	179 (99.5)	164 (91.5)	86 (47.8)	59 (32.8)	6 (3.3)	2 (1.1)	0 (0.0)	0 (0.0)
Chi-square		NA	0.052	1.752	0.052	33.948	30.481	0.912	0.253	NA	NA

Intervention (Malaria Treatment)	Total Number Examined	Post Intervention (Day 1 to 4)		Post Intervention (Day 5 to 9)		Post Intervention (Day 10 to 14)		Post Intervention (Day 15 to 19)		Post Intervention (Day 20 to 24)	
		HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)	HRP2 (%)	HRP3 (%)
Df		NA	1	1	1	1	1	1	1	NA	NA
p Value		NA	0.819ns	0.186ns	0.819ns	<0.001*	<0.001*	0.340ns	0.615ns	NA	NA

Keywords; HRP=Histidine Rich Protein, Df=Significance different, NA=Not average, ns=not significance, <=less significant.

Table 4 presents the correlation between selected biofactors and antimalarial drug resistance. The analysis revealed a positive correlation between all three biofactors and resistance patterns. Genetic marker PFC RT showed the strongest correlation with resistance $r = 0.65$, followed by

Anemia $r = 0.50$ and HIV $r = 0.45$. This indicates that genetic mutations, poor nutritional status, and comorbidities may contribute to reduced drug efficacy and delayed parasite clearance in the study area.

Table 4: Associated Biofactors with Persistent Positivity of Antigen of RDTs used after Antimalarial Drug Administration

Biofactors	Correlation with resistance (r)
Genetic marker (PFCRT)	0.65
Anemia	0.50
HIV	0.45

Table 5: Correlation Between Biofactors and Persistent Positivity of RDT Antigen

Biofactor	Correlation Coefficient r	Strength of Association	Interpretation
PfCRT Genetic Marker PFC RT	$r = 0.65$	Strong positive	Strongest predictor of persistence
Anemia	$r = 0.50$	Moderate positive	Anemia increases persistence
HIV Co-infection	$r = 0.45$	Moderate positive	HIV increases persistence
Malnutrition	$r = 0.42$	Moderate positive	Poor nutrition delays clearance
Age >30 years	$r = 0.38$	Weak positive	Older age slight delay

Pearson correlation, $p < 0.05$ for all. PfCRT mutation showed strongest correlation $r = 0.65$ with persistence positivity.

Discussion

This study assessed *Plasmodium falciparum* clearance using Histidine Rich Protein 2 and Histidine Rich Protein 3 Rapid Diagnostic Tests among Infants and Lactating Mothers in Dutsin-Ma, Dutsin-Ma Local Government Katsina State. The overall prevalence of 59.2% observed in this study is high and indicates that malaria remains endemic in Dutsin-Ma. This finding is in agreement with the World Malaria Report which reported that Nigeria accounts for 27% of global malaria burden (WHO, 2023). The high prevalence is also similar to the 60.3% reported by Okonkwo *et al.*, (2021) among mothers and children in Sokoto, Northwest Nigeria, and 58.7% reported by Abubakar *et al.*, (2022) in Katsina State. However, it is higher than 41.2% reported by Bassey *et al.*, (2020) in Calabar and 36.5% reported by Anumudu *et al.*, (2019) in Ibadan, Southwest Nigeria. The differences may be due to geographical variation, seasonal transmission, and ecological factors. Dutsinma lies in Sudan savanna with prolonged rainy season from May to September which favours Anopheles breeding (Abaje *et al.*, 2014). Poor drainage, open water storage and low utilization of Long Lasting Insecticidal Nets (LLNs) among lactating mothers may also contribute (Snow *et al.*, 2017; FMOH, 2020). The high persistence 99.4% in infants on Day 5-9 is concerning because WHO 2009 recommends >90% parasite clearance by Day 3 for effective antimalarial (WHO, 2009; WHO, 2015). The persistence of HRP2 46.7% among infants on Day 14 agrees with work of Ly *et al.*, 2010 in Mali who reported 48% HRP2 positivity on Day 14, and Swarthout *et al.*, (2007) in DR Congo have being reported 52% persistence at Day 14 among under-five. However, it is higher than 21%

reported by Karbwang *et al.*, (2019) in Southeast Asia. The persistence is explained by the Biology of HRP2 antigen. HRP2 is a stable protein released from infected RBCs and can persist in blood for 2-4 weeks after parasites have been cleared by drugs Murray *et al.*, 2018. The significant age effect $p < 0.001$ where older infants >2.0 years had 83.3% positivity vs 25.0% in younger infants 0-0.3 years in Table 4.2. this finding was in line with the work of Okell *et al.*, (2012) and Reynaldi *et al.*, (2019). Younger infants have maternal antibodies and lower parasite biomass, leading to faster clearance. Older infants have waned maternal immunity and may have higher parasite density requiring longer clearance Moormann, (2009). The study showed 99.5% persistence in Table 3. among lactating mothers is similar to findings of Tiono *et al.*, (2014) in Burkina Faso who reported 96% among postpartum women on Day 7, and Swarthout *et al.*, (2007) who reported 84% on Day 7 in DR Congo. It is higher than Houze *et al.*, 2011 who reported 38% on Day 7 in non-endemic setting. The high rate in Dutsin-Ma may be due to high initial parasite biomass, altered pharmacokinetics during lactation, and low immunity (Kourtis *et al.*, 2014; Sholapurkar *et al.*, 2018). The 47.7% positivity on Day 14 in Table 2.1. agrees with the work of Singh *et al.*, (2020) in India who found that 48.5% among lactating mothers on Day 14, and Ly *et al.*, (2010) in Mali that found 51% among women. The drop to 3.4% on Day 19 and 0% on Day 24 shows eventual complete antigen clearance, which confirms that treatment was ultimately effective and agrees with Tjitra *et al.*, (2001) and Abeku *et al.*, (2008) in Ethiopia and Indonesia that reported 0-2% on Day 21. Age significantly affected clearance $p < 0.001$. Mothers 21 years

cleared fastest 25.8% still positive on Day 14 while mothers >40 years had 85.7% still positive. This is consistent with Okell *et al.*, (2012) and Kayode *et al.*, (2020) reported that older individuals have reduced immunity and slower drug metabolism leading to delayed antigen clearance. Younger mothers may have better immune response and lower parity-related immunosuppression Diala *et al.*, (2017) and Udomah *et al.*, (2021). This contradicts Fana *et al.*, 2020 who found younger mothers had higher persistence in Zamfara, indicating geographical variation. Lactating mothers treated with ACT had 63.0% HRP2 persistence vs 18.0% for Fansidar $p < 0.001$. This is paradoxical because ACT Artemether-Lumefantrine is expected to clear parasites faster than SP (White, 2018) and (FMOH, 2020). The artemisinin component clears 10,000-fold parasite biomass in 48h, while SP has long half-life but high resistance (Atroosh *et al.*, 2012; Adebobola, 2021). Delayed ACT clearance is an early warning of artemisinin partial resistance reported in Rwanda (Uwimana *et al.*, 2020; Uganda Balikagala *et al.*, 2021; Eritrea Berhane *et al.*, 2023). The mechanism involves *kelch 13* R561H, C469Y mutations and PfCRT K76T (Fidock *et al.*, 2000; Ariey *et al.*, 2014). However, in lactating mothers, other factors may contribute: Fear of drug transfer to breast milk leads to poor adherence and does sharing (Ariey *et al.*, 2019), administration without fatty food reduces absorption by 10-fold (Koren *et al.*, 2008; White, 2008), and altered volume of distribution during lactation may lower drug levels (Koren *et al.*, 2015). Circulation of fake ACTs in Dutsin-ma markets may also explain (FMOH, 2020). HRP3 showed lower positivity 92.1% baseline vs 100% HRP2, 32.7% vs 47.7% on Day 14. This agrees with Baker *et al.*, (2005) and Lee *et al.*, (2012) that HRP3 is expressed at lower levels and shares epitopes with HRP2 but has lower sensitivity. Deletion of *pfrp2/pfrp3* genes reported in Nigeria Oguike *et al.*, (2018) may also affect pattern.

The strongest biofactor was PfCRT gene mutation with $r=0.65$, and 69.0% of those with mutant PfCRT had persistent HRP2 in Table 4.4 vs 33.5% in wild type $p=0.001$. This aligns with previous studies that identified mutations in *pfcrt* gene at codon K76T as key determinant of chloroquine resistance and reduced susceptibility to amodiaquine, a partner drug in ACT (Fidock *et al.*, 2000 and Adebobola, 2021). PfCRT is a transporter in food vacuole membrane that effluxes drug away from target. Mutant PfCRT reduces drug accumulation leading to slow parasite clearance and prolonged antigenemia (Ecker *et al.*, 2006; Valderramos and Fidock, 2006). Similar strong correlation $r=0.68$ was reported by Atroosh *et al.*, (2012) in Malaysia and $r=0.61$ by Okonkwo *et al.*, (2021) in Sokoto, Nigeria. The presence of PfCRT mutation in 39.4% in Dutsin-Ma suggests that parasites with reduced susceptibility circulate in the area, which may explain the delayed ACT clearance 63.0% observed. This is supported by reports of PfCRT K76T prevalence of 45% in Katsina by Abubakar *et al.*, (2022). Anemia showed moderate positive correlation with persistent positivity. 59.6% of anemic participants had persistent HRP2 vs 32.7% non-anemic $p=0.001$. This agrees with studies by Sowunmi *et al.*, (2006); Buffet *et al.*, (2009) and Price *et al.*, (2019) reported that anemia impairs drug absorption, immune response and parasite clearance. Anemic individuals have reduced red cell mass, altered pharmacokinetics of lumefantrine, and poor immune-mediated clearance of parasitized RBCs (Okell *et al.*, 2005; White, 2018; Sholapurkar *et al.*, 2018). In infants, anemia reduces oxygen delivery and effects splenic clearance of HRP2-containing RBCs (Gosling *et al.*, 2014). Similar correlation $r=0.52$ was reported by Elechi *et al.*, (2022) in Maiduguri among children and $r=0.48$ by Bassey *et al.*,

(2020) in Calabar. HIV positivity showed persistent antigen. 72.2% of HIV positive participants had persistent HRP2. This is consistent with findings of Korenromp *et al.*, (2005) and Cuadros *et al.*, (2019) reported that HIV immunosuppression increases susceptibility to malaria, reduces treatment outcome and delays parasite clearance. Malnutrition showed high persistent positivity among study infants. Malnutrition affects drug absorption, protein binding of lumefantrine and immune response (Bhutta *et al.*, 2005 and Shikur *et al.*, 2016). Malnourished infants have impaired splenic function and longer HRP2 circulation (Mouatcho and Goldring, 2013). These biofactors do not cause RDT false positivity, but cause true delayed parasite clearance which results in prolonged HRP2 antigenemia PfCRT mutation causes intrinsic resistance, while anemia, HIV and malnutrition cause host-related reduced drug response (Fresno *et al.*, 2002 and Ochola, 2011). Therefore, persistent positivity on Day 14 in Dutsin-Ma is likely a combination of parasite genetic factor strong plus host biofactors as proposed by the WHO 2023 framework for antimalarial resistance.

The study revealed an overall prevalence of 213 (59.2%) out of 360 participants were positive. This indicates that malaria due to *P. falciparum* remains highly endemic in Dutsin-ma Local Government Area and constitutes a major public health problem among the vulnerable groups. The prevalence is far above the National Malaria Strategic Plan target of <10% by 2025 FMOH, 2020, showing that current control interventions are inadequate in the study area.

The study determined that HRP2 and HRP3 antigens show a persistent positivity pattern among infants after antimalarial treatment characterized by high persistence on Day 5-9 99.4%, moderate persistence on Day 10-14 46.7% and complete clearance 0% by Day 20-24. Age significantly influenced clearance pattern, with older infants showing delayed clearance. Also RDT should not be used alone to assess treatment failure in lactating mothers on Day 14. Microscopy should be mandatory after Day 3 WHO, (2009). Also the study shows pattern of slow clearance, this slow clearance is consistent with the known stability of HRP2 protein. HRP2 persists in blood for 2-4 weeks after parasite death and therefore RDT remains positive even when parasites are cleared Among 180 lactating mothers, HRP2 and HRP3 antigens showed persistent clearance pattern with 100% at baseline, 99.5% on Day 5-9, 47.7% on Day 10-14, 3.4% on Day 15-19 and complete clearance 0% by Day 20-24. Older lactating mothers >40 years had significantly delayed clearance 85.7% vs 25.8% in younger mothers 19-21 years. Mothers treated with ACT had significantly delayed clearance 63.0% Fansidar on Day 10-14, which warrants urgent molecular surveillance for artemisinin resistance in lactating population.

The study identified that biofactors are significantly associated with persistent positivity of RDT antigen after antimalarial treatment. PfCRT K76T genetic mutation was the strongest associated biofactor $r=0.65$ $p=0.001$ with 69.0% persistence among mutant's vs 33.5% wild type. This was followed by anemia $r=0.50$, 59.6% vs 32.7%, HIV co-infection $r=0.45$, 72.2% vs 44.8% and malnutrition $r=0.42$, 63.4% vs 40.3%. This indicates that persistence antigen positivity in Dutsin-ma is driven by both parasite resistance markers and host vulnerability factors, which together contribute to delayed parasite clearance and prolonged HRP2/HRP3 antigenemia up to Day 14.

CONCLUSION

The study revealed an overall *Plasmodium falciparum* prevalence of 59.2% among lactating mothers and infants

attending General Hospital Dutsin-Ma, with significantly higher prevalence in lactating mothers 68.9% compared to infants 49.4%. This prevalence far exceeds the National Malaria Strategic Plan target of <10% by 2025 (FMOH, 2020) and confirms that malaria remains highly endemic in Dutsin-Ma.

Assessment of HRP2 and HRP3 antigen clearance showed a characteristic persistence pattern: 100 on at baseline Day 1-4, 99.4% on Day 5-9, 46.7% among infants and 47.7% among lactating mothers on Day 10-14, 3.9% on Day 15-19 and complete clearance 0% by Day 20-24. This pattern is consistent with the known stability of HRP2 protein which persists for 2-4 weeks after parasite death (Dalrymple et al., 2018). Age significantly influenced clearance ($p < 0.001$), with older infants >2.0 years (83.3%) and older lactating mothers >40 years (85.7%) showing delayed clearance compared to younger groups (25.0% and 25.8% respectively).

Paradoxically, participants treated with ACT had significantly higher persistent positivity on Day 10-14 (HRP2 63.0%, HRP3 46.2%) compared to Fansidar group (18.0% and 6.6%, $p < 0.001$). While eventual clearance by Day 20-24 indicates treatment was ultimately effective, this delayed ACT clearance is concerning and may indicate reduced ACT efficacy or tolerance requiring urgent molecular surveillance for kelch13 and PfCRT markers (Balikagala et al., 2021).

Biofactors analysis revealed PfCRT K76T mutation as the strongest predictor of persistence ($r = 0.65$, $p = 0.001$; 69.0% persistence in mutant's vs 33.5% wild type), followed by anemia ($r = 0.50$, 59.6% vs 32.7%) and HIV co-infection ($r = 0.45$, 72.2% vs 44.8%). This indicates that persistent RDT positivity in Dutsin-Ma is driven by both parasite resistance markers and host vulnerability factors as proposed by WHO (2023) framework.

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