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Parasite Gene Expression and Evidence of Emerging Lonart Resistant *Falciparum* Malaria Infection in Patients in Benin Metropolis, South-South, Nigeria

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ABSTRACT: This study investigated the possible emergence of *Plasmodium falciparum* malaria resistance to Lonart, a drug used in the treatment of malaria. The expression of the PFK13 gene, known for its association with resistance to artemisinin, was examined. One hundred patients who had a relapse of malaria, after treating with Lonart, were recruited for this study. The patients were treated with Quinine following their relapse. Blood samples were collected before, and 2 weeks after treatment with Quinine Malaria parasite (MP) and full blood count (FBC) tests were performed and compared with a Control group. The expression of the PFK13 gene of the *plasmodium falciparum* parasite in the blood of these patients, was also investigated. The results show that the MP test was positive, while the FBC of patients, before their treatment with Quinine, was significantly ($p<0.05$) lower than that of the Control. The MP test was negative, with no significant difference ($p>0.05$) in the FBC of the patients, after their treatment with Quinine, compared to the Control. The PFK13 gene was significantly expressed ($p<0.05$) in patients who had a relapse after treatment with Lonart, compared to the Control. This study revealed the potential emergence of Lonart resistant malaria in Benin Metropolis.

Keywords: Malaria parasite, Lonart, Drug resistance, PFK13 gene expression, Full blood count.

Introduction

Malaria has been recognized as a severe health concern since ancient times, and it continues to be one of the world's most dangerous illnesses, with high morbidity and mortality rates in people and other animals (Andrews *et al.*, 2014). *Plasmodium falciparum* infections contribute to over 90% of global malaria-related deaths, representing a significant public health burden (Snow, 2015).

An infected female Anopheles mosquito is the primary vector for humans, infecting them with sporozoites while feeding on blood. The same species of anopheles mosquitoes also act as a vector for transmission. Malaria could be fatal if untreated due to the disruption of the blood supply to vital organs (Milner, 2018). Human *Plasmodium* species share a common life cycle, commencing with liver development and concluding with subsequent proliferation in the host's bloodstream. Haematological changes associated with malaria infection exhibit variability influenced by factors such as the level of malaria endemicity, background haemoglobinopathy, demographic variables, and the individual's malaria immunity. The ability to predict these haematological alterations is crucial for clinicians to implement effective and early therapeutic interventions, thereby preventing the occurrence of major complications. White blood cell counts during malaria typically range from low to normal, reaching their minimum levels around the onset of fever and when the infection becomes detectable through microscopy (Giribaldi *et al.,* 2014). White blood cell counts have been used to estimate parasitaemia microscopically, and automated detection of malaria pigments in white blood cells has been employed for

malaria diagnosis. Regardless of the method, monitoring the trend of white blood cell counts over time and with treatment can provide valuable guidance for managing the disease (Giribaldi *et al.,* 2014).

Certain antimalarial drugs such as quinine, chloroquine, and artemisinin, have been employed in the treatment of malaria. Treatment of *Plasmodium falciparum* malaria is heavily reliant on artemisinin based combination therapy. Presently, no replacement antimalarial drugs with safety and efficacy profiles comparable with ACTs are available. Thus, a major health crisis is feared if the current decreased susceptibility phenotype progresses to full-blown resistance and/or becomes widespread in Africa, where most deaths occur. Resistance to antimalarial drugs has become a significant obstacle in the efforts to control malaria, and its prevalence is surpassing the rate at which new antimalarial drugs are being developed for practical clinical application. (WHO, 2020).

Artemisinin resistance in clinical settings, characterized by delayed parasite clearance, was initially identified in Pailin, Western Cambodia, in 2009 (Dondorp *et al.,* 2017). This resistance is predominantly influenced by mutations in the propeller domain of *Plasmodium falciparum* chromosome 13 (K13-propeller) in Southeast Asia. (WHO, 2019b, Straimer *et al.,* 2021). The development of resistance to artemisinin-based combination therapy (ACT) partner drugs has led to treatment failures with various ACTs, including artemether-lumefantrine, artesunate-mefloquine, and artesunate-amodiaquine (Menard and Dondorp, 2017; Leang *et al.,* 2013). It is therefore essential to systematically monitor the emergence and spread of resistance to artemisinin and partner drugs. This surveillance is crucial for informing public health interventions and ensuring appropriate administration at the Local Government level. With the increase levels of malaria recrudescence in the Benin metropolis, there was therefore, a need to study the potential emergence of resistance to artemisinin and partner drug by the *Plasmodium falciparum* parasite in Benin City, Nigeria.

Materials and methods

Study location and setting: A facility-based cross-sectional study was conducted using patient at Water-gate Medical Center, GRA, Faith Mediplex Hospital, GRA and Sama Poly Clinic 1st East Road, all in Benin City, Edo State, Nigeria.

Sample size determination: Sample size was calculated from the Cochran's formulae (Cochran, 1977), standard deviation was set at 1.96, prevalence rate of 8%, precision level of 5%. Accordingly, the calculated sample size was 124.

$$\frac{N =}{d^2} \quad Z^2 pq$$

where N = sample size
  Z = Standard deviation set at 1.96
  p = Prevalence of malaria
  q = 1-p (Prevalence of malaria)
  d = Precision level = 0.05

The prevalence of artemisinin resistance 3.5-8%
q = 1-p = 1-8%
q = 1-0.08 = 0.92

$$N = \frac{(1.96)^2 \text{x } 0.08 \text{ x } 0.92}{(0.05)^2} \quad = 113.1$$

Sample size (N) =113

10% Attrition ratio = 11.3
Sample size = 113+11= 124
Sample size = 124 samples

Inclusion criteria: Of the 124 sample size recruited for this study, only 100 met the inclusion criteria. Informed consent and the presence of *P. falciparum malaria,* as confirmed by laboratory data (parasite count ranging from 2,000 to 200,000 per μl blood) two weeks following treatment with artemisinin-based combination therapy (Lonart for this study, composed of artemether and lumefantrine), were prerequisites for inclusion into the study population for this study.

 Other inclusion criteria were:
1. The ability to be monitored for 7 days following treatment with artemisinin-based combination therapy, and the willingness to return for the 14th day checkup.

2. The presence of febrile symptoms induced by *Plasmodium falciparum* malaria, following the malaria febrile pattern, and not febrile patterns or fever from other infections.
3. Lack of general risk signs or symptoms of severe and complicated P. falciparum malaria.
4. The fact that the patient had artemisinin-based combination therapy, (Lonart in this study) and is still experiencing malaria symptoms two weeks later.
5. Febrile patients with clinical suspicion of malaria who were at least 5 years old and consented to take part in the research.

Exclusion criteria

The study excluded:

1. Patients with clinical symptoms consistent with severe or complicated malaria,
2. Patients with symptoms or signs unrelated with malaria.
3. Patients who experienced recurrent vomiting, diarrhea, malnutrition, pregnancy, resistance to medication, or failed to attend the required follow-up appointments on days 7 and 14.

Informed consent and ethical clearance: Informed consent was taken from the patients and parents of minors, after explaining to them the research protocol and making them understand what was expected of them. Prior to the commencement of the study, ethical clearance with number CMS/REC/2023/351, was requested, approved and obtained from the research ethics committee, College of Medical Sciences, University of Benin, Benin City, Nigeria.

Sample collection: Blood sample for malaria parasite and full blood count was collected into EDTA sample bottles. Venous blood (2 Ml) was utilized for these procedures. Blood sample for PFK13 gene expression was collected in a plain sample bottle containing DNA/RNA shield in the ratio of 1:1 and left to stand at room temperature until further analysis

Laboratory procedure: Malaria parasite test was carried out microscopically to confirm the presence of malaria using the thin and thick blood smear method. Blood slides were prepared and stained with 10% Giemsa stain. Microscopic abnormality of blood in smear and presence or absence of malaria was determined. All malaria positive blood samples and those of the control group were analyzed for blood cell counts. Blood counts were performed using an automated haematology analyzer, the Beckman Coulter counter (URIT-3300) (Beckman Coulter. 1947), following the manufacturer's instructions. The following haematological parameters were considered for this study: percentage of packed cell volume (PCV %), white blood cell counts (WBC), percentage of lymphocytes (LYM %) eosinophils (EOS cm^3), and percentage of neutrophils (NEUT %).

Malaria parasite test: The thick and thin blood films stained with Giemsa's stain which is the gold standard for diagnosing malaria (WHO 2010, Norgan *et al.,* 2013) was used for this procedure. A small amount of peripheral blood was drawn from the patient's finger and placed on a glass slide. Thick and thin blood film was made using another slide as spreader, thereafter, it was the air dried for 15 minutes. The thick blood smears are used to detect the presence of plasmodium parasite while the thin blood smears are used to identify the Plasmodium species causing the infection and the developmental stages that are currently circulating in the patient's blood. The film was fixed by dipping into methanol for 1 minute and then stained with Giemsa's stain 10 seconds. The slide was washed using clean water and was allowed to dry. The dried slide was then viewed under the light microscope x 40 0bjective for the presence of malaria parasite and also for the stage of the parasite

Treatment and post-treatment laboratory analysis: On the day the patients reported sick to the hospital after their initial treatment with Lonart and a suspicion of Malaria parasite infection was made, the first sample collection was done. The patients were treated with Quinine infusion and tablets for a combined total period of 5 days. Patient returned for follow-up visit on day 7 and those that were afebrile asked to return on day 14. Blood samples were collected for post Quinine treatment malaria parasite test and analysis of full blood count parameters on day 14, to confirm their full recovery.

Parasite genomic DNA extraction: Blood samples were analyzed for parasite genomic DNA extraction, in accordance with the manufacturer's protocol using the Zymo Quick-DNA Miniprep Plus kit Murphy Avenue, Irvine, California, United States of America).

Amplification of Pfk13 gene: The Pfk13 propeller domain was amplified by nested PCR. The primers utilized for the primary PCR were kelch-outer-F 50-gggaatctggtggtaacagc-30 and kelch-outer-R 50-cggagtgaccaaatctggga-30. For the nested PCR, the primers were kelch-inner-F50-gccaagctgttgaaagaagcaga-30 and kelch-inner-R 50-gccaagctgccattcatttg-30. The 849-bp nested PCR product will correspond to codons 427–709 of the PF3D7_1343700 K13 propeller domain, which contain mutations associated with delayed parasite clearance (representing nucleotide sequence 1279–2127). In the primary reaction, the PCR solution was reconstituted to a final volume of 20 μL. For amplification, 2 μL of DNA was subjected to 0.5 μM of each primer. The cycling conditions consisted of 95˚C for one minute, followed by 35 cycles at 60˚C for one minute, 58˚C for 20 seconds, and 95˚C for 20 seconds; the final extension lasted for three minutes at 60˚C. An additional 0.5 M of each primer was utilized to amplify one microlitre of the primary reaction in the nested PCR procedure. The cycling regimen consisted of an initial 5 minutes at 95 ˚C, followed by 35 cycles at 56 ˚C for 20 seconds, 95 ˚C for 20

seconds, and 60℃ for 1 minute; the exercise concluded with a final extension at 60℃ for 3 minutes. To validate amplification, nested amplicons were subjected to electrophoresis on a 2% agarose gel. The band density was quantified using ImageJ[TM] software.

Statistical analysis: Statistical package SPSS version 20 (IBM corporation, NY) for windows was used to analyze the data obtained from this study. Results obtained were expressed as mean ± SEM (Standard Error of Mean). Differences among the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant if *p*-value is less than 0.05 (*p*< 0.05). LSD Post Hoc test was used to determine where the significance lay.

Results

Full blood count of patients with Lonart resistant malaria: In Table 1, there was a significant reduction (*p*<0.05) in the PCV value before treatment compared to the control group, but there was no significant reduction (*p*>0.05) in the PCV value after treatment compared to the control group. There was also a significant reduction (*p*<0.05) in the WBC count before treatment compared to the control group, but the WBC count came back to normal after treatment as there was no significant difference (*p*>0.05) in their values, when compared to the control group. There was no significant difference (*p*>0.05) in the values of lymphoctyes, eosinophils, and neutrophils before treatment and after treatment, when compared to their respective control groups.

In Table 2, for the male gender, there was significant reduction (*p*<0.05) in the PCV value before treatment compared to the control group, but there was no significant reduction (*p*>0.05) in the PCV value after treatment compared to the control group. There was also a significant reduction (*p*<0.05) in the WBC count before treatment compared to the control group, but there was no significant difference (*p*>0.05) in the WBC count after treatment compared to the control group. There was also no significant difference (*p*>0.05) in the values of neutrophils, lymphocytes and eosinophils, before treatment and after treatment. In the female gender, there was also a significant reduction (*p*<0.05) in the PCV value before treatment compared to the control group, but there was no significant difference in the PCV value after treatment compared to the control group. There was no significant difference (*p*>0.05) in the WBC count and its differentials before and treatment when compared to their respective control groups.

Across the three age groups, as shown in table 3, there was a significant reduction (*p*<0.05) in the PCV value before treatment compared to the control group, but there was no significant difference (*p*>0.05) in the PCV value after treatment compared to the control group. There was some reduction but there was no significant difference (*p*>0.05) in the WBC value and its differentials, before and after treatment for all age groups, when compared to the control group (Table 3), except for those over 40yrs whose WBC count was significantly reduced (*p*<0.05), when compared to the value of its control.

In Table 4, there was a significant difference (*p*<0.05) in the expression of PFK13 gene in patients with Lonart resistant malaria compared to the control groups.

In figure 1a to figure 1d, the gel electrophoresis which reveals the migration of the PFK13 gene in the test and control group from the negative pole to the positive pole is shown. It represents the band intensity of PFK13 gene expression in subjects with Lonart resistant malaria compared with the control groups. The test group has greater band intensity when compared with the control groups. Patients with Lonart resistant *Plasmodium falciparum* malaria showed higher expression of PFK13 gene compared to the control group.

Table 1: Full blood count of patients with Lonart resistant malaria before and after treatment

Groups	% PCV	WBC (cm^3)	% Neutrophils	% Lymphocytes	% Eosinophils
Control	49.86±0.41[a]	7512.42±206.60[a]	57.95±0.40[a]	40.03±0.60[a]	3.02±1.04[a]
Before Treatment	30.26±0.17[b]	6269.00±116.49[b]	58.92±0.11[a]	39.09±0.10[a]	1.99±0.08[a]
After Treatment	43.89±0.37[a]	6850.00±120.60[a]	58.84±0.11[a]	39.11±0.09[a]	2.03±0.08[a]

Values are expressed as Mean±SEM. Means with different superscript alphabets in the same column are statistically significantly different from one another at *p*<0.05.

Table 2: Full blood count of patients with Lonart resistant malaria before and after treatment according to gender

Gender	Groups	% PCV	WBC (cm³)	% Neutrophils	% Lymphocytes	% Eosinophils
Male	Control	40.75±0.41[a]	7000.00±504.14[a]	58.18±1.66[a]	40.31±1.60[a]	2.68±0.17[a]
	Before Treatment	30.22±0.24[b]	6133.33±172.39[b]	58.71±0.18[a]	39.28±0.14[a]	2.00±0.12[a]
	After Treatment	41.24±0.30[a]	6986.66±180.97[a]	59.00±0.15[a]	39.04±0.14[a]	1.95±0.11[a]
Female	Control	39.69±0.49[a]	7014.78±227.72[a]	58.61±0.81[a]	39.78±0.65[a]	2.95±1.74[a]
	Before Treatment	30.29±0.24[b]	6680.00±157.72[a]	59.09±0.14[a]	38.92±0.15[a]	1.98±0.11[a]
	After Treatment	42.41±0.61[a]	6883.63±160.98[a]	58.70±0.16[a]	39.16±0.29[a]	2.09±0.12[a]

Values are expressed as Mean±SEM. Means in the same column of the same section, with different superscript alphabet, are statistically significantly different from one another at $p<0.05$.

**Table 3:** Full blood count of patients with Lonart resistant malaria before and after treatment according to age

Age	Groups	% PCV	WBC (cm³)	% Neutrophil	% Lymphocytes	% Eosinophils
≤20	Control	40.41±0.23[a]	6806.52±273.43[a]	59.30±0.91[a]	40.00±0.86[a]	1.76±0.18[a]
	Before Treatment	30.55±0.58[b]	6588.88±421.12[a]	58.66±0.40[a]	39.55±0.24[a]	1.77±0.27[a]
	After Treatment	43.44±2.74[a]	6566.66±359.78[a]	58.77±0.40[a]	39.22±0.27[a]	1.77±0.22[a]
21-39	Control	39.38±0.74[a]	7187.81±303.83[a]	59.12±0.37[a]	40.05±0.86[a]	1.50±0.22[a]
	Before Treatment	30.10±0.22[b]	6419.29±141.67[a]	58.89±0.15[a]	39.12±0.13[a]	1.98±0.11[a]
	After Treatment	41.92±0.45[a]	6366.66±148.47[a]	58.82±0.15[a]	39.07±0.13[a]	2.10±0.11[a]
≥40	Control	39.86±0.41[a]	7012.4±202.60[a]	59.95±0.40[a]	39.03±0.60a	2.02±0.04[a]
	Before Treatment	30.44±0.30[b]	5632.35±214.15[b]	59.02±0.18[a]	38.91±0.20[a]	2.05±0.14[a]
	After Treatment	41.41±0.31[a]	5976.47±216.23[a]	58.88±0.19[a]	39.14±0.15[a]	1.97±0.14[a]

Values are expressed as Mean±SEM. Means in the same column of the same section, with different superscript alphabets are statistically significantly different from one another at $p<0.05$.

Table 4: PFK13 gene expression (band intensity values) of patients with Lonart resistant malaria

| Negative Control	Positive Control	Lonart
109.78±18.38[a]	118.32±17.13[a]	143.94±6.85[b]

Values are expressed as Mean±SEM. Means in the same row of the, with different superscript alphabets are statistically significantly different from one another at $p<0.05$.
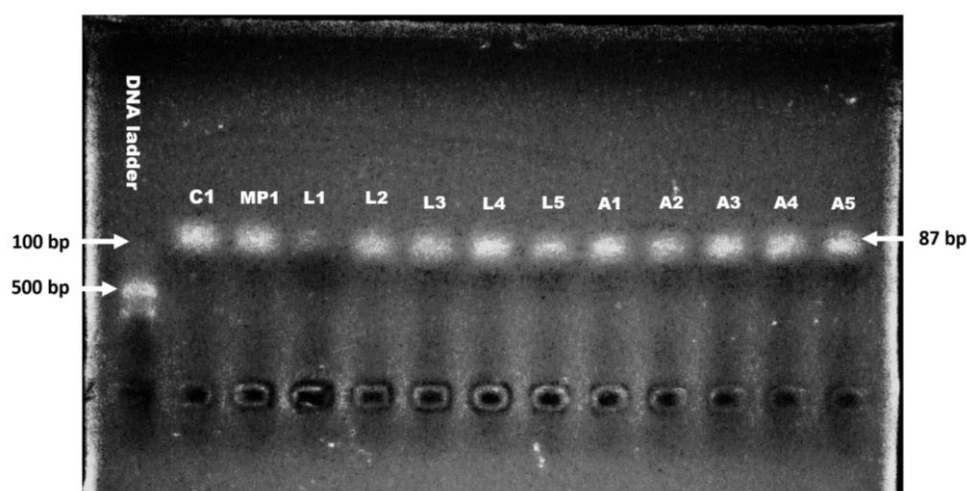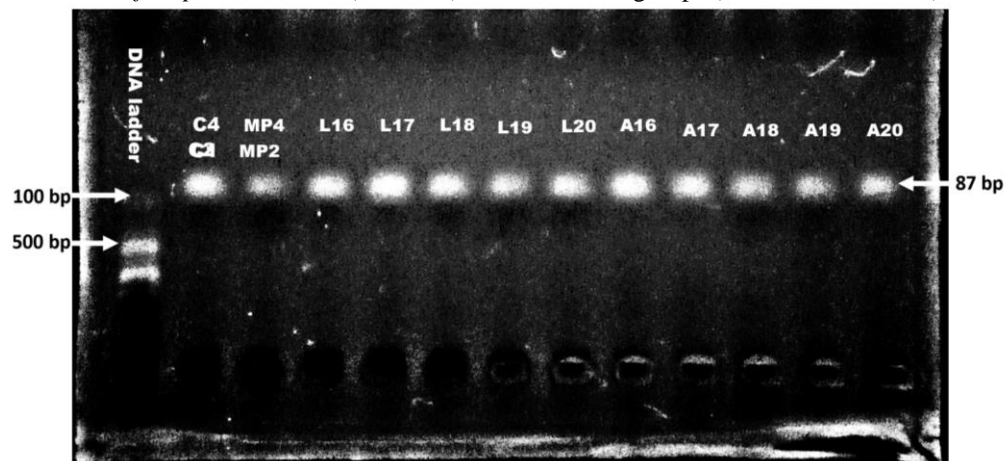

Figure 1a: Gel electrophoresis for the gene expression of PFK13 gene in Lonart resistant *Plasmodium falciparum* malaria (L1-L5) and the control groups (C1, MP1, A1-A5)
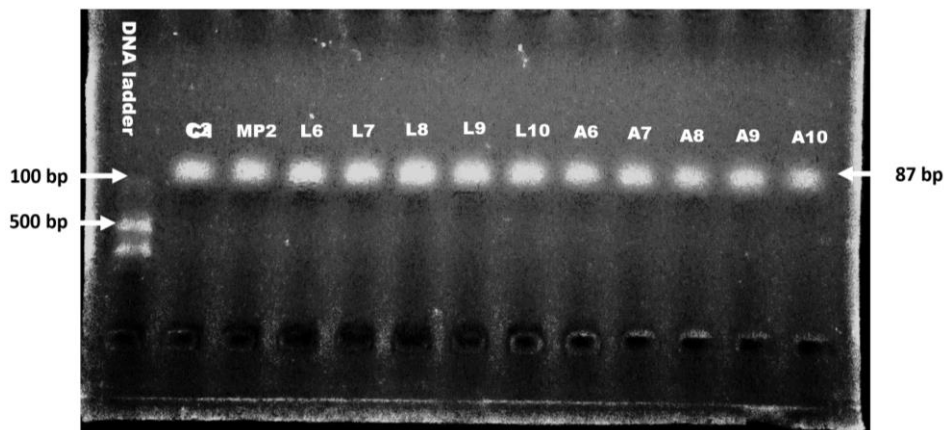
Figure 1b: Gel electrophoresis for the gene expression of PFK13 gene in Lonart resistant *Plasmodium falciparum* malaria (L6-L10) and the control groups (C2, MP2, A5-A10)
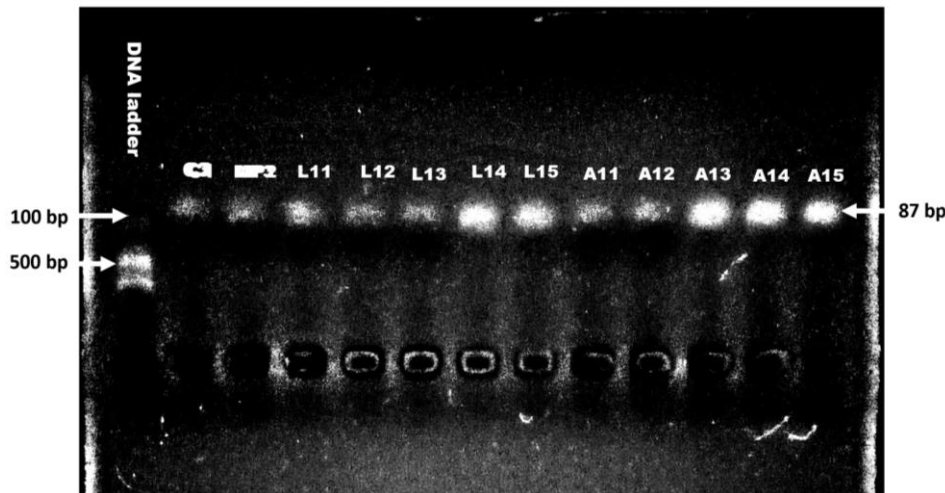
Figure 1c: Gel electrophoresis for the gene expression of PFK13 gene in Lonart resistant *Plasmodium falciparum* malaria (L11-L15) and the control groups (C3, MP3, A11-A15)

Figure 1d: Gel electrophoresis for the gene expression of PFK13 gene in Lonart resistant *Plasmodium falciparum* malaria (l15-l20) and the control groups (c4, mp4, a15-a20)

Key:
C = Malaria negative control group
MP = Malaria positive control group
L = Lonart resistant malaria group
A = Artequin resistant malaria control group

Discussion

Infection with the Malaria parasite, is a significant public health concern that often leads to changes in full blood count parameters. This plays a crucial role in malaria pathology, so data on full blood count parameters could serve as a marker for antimalarial drug resistance (Hänscheid *et al.,* 2008). Resistance to *Plasmodium falciparum*, one of the causative agent of malaria, is associated with mutations in its PFK13 gene, particularly in its propeller region, and so it is considered a molecular marker for artemisinin resistance (Inoue *et al.,* 2018,). Proteins associated with increased gene expression have been linked to various drug-resistant traits in *Plasmodium falciparum* (Calcada *et al.,* 2020). Gene expression, which can influence protein levels, is a potential mechanism for modulating drug responses. However, there have been limited studies investigating the patterns of gene expression in *Plasmodium falciparum* during artemisinin-based combination therapy (ACT) for uncomplicated malaria cases.

Notably, while artemisinin resistance is primarily linked to an increase in total PFK13 gene expression, this increase is actually due to an increase in mutated PFK13 gene fraction because there is usually decreased levels of normal PFK13, due to specific genetic mutations that has occurred in the original PFK13 gene (Birnbaum *et al.,* 2020). Lower levels of normal PFK13 transcripts in vivo have also been associated with prolonged parasite clearance times during artemether-lumefantrine (AL) treatment (Silva *et al.,* 2022).

In this study, full blood count parameters and parasite's PFK13 gene expression were analyzed in subjects with resistance to Lonart in Benin metropolis. The results highlighted changes in full blood count parameters and parasite's PFK13 gene expression before and after treatment with Quinine infusion (an alternative antimalarial drug), providing valuable insights into artemisinin resistance.

Subjects with Lonart resistance displayed similar variations in white blood cell (WBC) and packed cell volume (PCV) values. Before Quinine treatment, PCV values were significantly (P<0.05) lower, indicative of excessive red blood cell destruction. After Quinine infusion, however, values normalized, aligning with previous findings (Meraiyebu and Ajibola, 2012). White blood cell values were consistently significantly lower (P<0.05), consistent with reports by McKenzie *et al.,* (2005), stating *Plasmodium falciparum* malaria patients' exhibit lower WBC counts. The mere fact that many patients were found who still tested positive to the malaria parasite despite treatment with Lonart and who subsequently recovered after treatment with Quinine, is indicative of evidence of the emergence of Lonart resistant malaria.

In both gender and across the age ranges, similar trends were observed in full blood count parameters. The PCV was significantly (P<0.05) lower before treatment with Quinine when compared to the control group while the values showed no significant difference after treatment with Quinine when compared to the control group. The WBC showed significantly (P<0.05) lower values too, but its differentials showed no significant difference (P>0.05).

The Greater Mekong subregion, is considered the focal point for the emergence of *Plasmodium falciparum* malaria resistance, with the potential for this resistance to spread to other malaria-endemic regions. Several factors contribute to this regional specificity and resistance to malaria treatment. This includes the varying levels of host immunity leading to frequent use of antimalarial drugs, genetic factors of the parasite stemming from its origins, limited access to effective drugs, and continued use of ineffective monotherapy. Additionally, the prevalence of low-quality and counterfeit antimalarial drugs is widespread, particularly in Sub-Saharan Africa, and is seen as a major factor driving the development and spread of resistance (Mita *et al.,* 2009).

From a molecular perspective, the emergence of resistance to artemisinins, a key antimalarial drug, is heavily influenced by the genetic background of the parasite. Resistance to artemisinins is known to be heritable and therefore has a clear genetic basis (Phyo *et al.,* 2012). Studies on genome modification have demonstrated that the impact of various mutations in the PFK13 gene on parasite clearance and survival rates depends on the genetic makeup of the parasites (Straimer *et al.,* 2015). Additionally, research by Lee and Fiddock (2016) suggests that mutations in certain DNA repair genes, such as mlh1, pms1, and exo1, are overexpressed in artemisinin-resistant parasites, further highlighting the role of genetics in resistance development.

Studies by Dahal *et al.* (2016) propose that monitoring for artemisinin resistance in Sub-Saharan Africa should consider a threshold of 5% of cases still positive for parasites on day 3, reflecting the higher levels of acquired immunity in African populations, which contribute to faster parasite clearance. Moreover, various factors can influence parasite clearance times during artemisinin-based combination therapy, including initial parasite load, efficacy of partner drugs, patient age, health status, and artemisinin dosage. Recent research indicates that lower levels of immunity are associated with a higher prevalence of Pfk13 mutations, suggesting that these factors should be integrated into monitoring efforts for artemisinin resistance (Thu *et al.,* 2017).

The gene expression results of this current study, showed that subjects with Lonart resistant malaria exhibited increased level of PFK13 gene expression compared to the control groups. There was a statistically significant increase (P< 0.05) compared to the positive and negative control groups. The level of PFK13 gene expression was similar when compared to the Artequin resistant malaria control group, suggesting that the pattern of gene

expression is similar in all artemisinin based antimalarial resistant group in this region. While mutations in the PFK13 gene are the primary genetic markers for artemisinin resistance, variations in gene expression could potentially influence the overall response of the parasite to artemisinin. However, high levels of PFK13 expression alone are not a direct indicator of Lonart resistant parasite. Sequencing the PFK13 gene provides more reliable indicator of Lonart resistant parasite. Therefore, molecular surveillance of these mutations would give a more accurate indication of Lonart resistant parasite rather than relying solely on the level of gene expression (She *et al.,* 2020). As part of strategies devised to contain the spread of artemisinin resistance, active monitoring of parasite responses and markers of artemisinin resistance in different areas is advocated as regional differences in parasite response may exist in Nigeria (Ikeda *et al.,* 2018). A potential challenge would be to identify and validate resistance markers if unique markers exist in other areas (Dokumu *et al.,* 2018).

This study contributes to understanding Lonart resistant parasite dynamics and emphasizes the need for continuous surveillance, including molecular analysis of resistance markers. Limitations of this study include the inability to identify PFK13 mutations; future studies should however, address this to validate the emergence of Lonart resistant *plasmodium falciparum* parasite in Benin metropolis.

Conclusion

Plasmodium resistance to anti-malarial drugs remains one of the most persistent issues in the effort to eradicate malaria. Regular monitoring of anti-malarial medication efficacy is critical for detecting changes in treatment effectiveness and permitting rapid responses to combat resistance and prevent its spread. This study was aimed at evaluating the prevalence of Lonart-resistant malaria infection in Benin City by examining the present state of the PFK13 gene expression, which has been recognized as a molecular indicator of artemisinin-resistant malaria. The investigation focused on changes in full blood count parameters and PFK13 gene expression associated with artemisinin resistance. This study revealed a persistent negative alteration in the full blood count parameters of subjects with Lonart resistant *Plasmodium falciparum* malaria despite having been treated earlier with this drug, which indicates that resistance to Lonart treatment exists. Furthermore, PFK13 gene expression was found to be significantly elevated in people who were resistant to Lonart. These results indicate the emergence of resistance to available artemisinin based anti-malarial medications, especially Lonart in this case.

To establish the functional role of PK13 mutation as molecular markers of Lonart resistant parasite, however, a specific mutation in the PFK13 gene is required. Therefore, a comprehensive examination of the kelch13 gene mutation in *Plasmodium falciparum* is imperative to establish its epidemiological impact on Lonart resistant parasite in Benin metropolis. Though this research gives evidence of the emergence of Lonart resistance malaria, further studies on the mutated PFK13 gene involved, will deepen our understanding of the dynamics of artemisinin resistance and help devise practical solutions to the ever-changing problems associated with treating malaria.

Institutional review board/ethical clearance

The study was approved and given ethical clearance by the Research Ethics Committee (REC) of the College of Medical Sciences, University of Benin. The REC Approval Number being: CMS/REC/2023/485.

Conflict of interest

The authors declare no conflict of interest.

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