Plasmodium falciparum histidine-rich protein 2 and 3 gene deletion in the Mount Cameroon region

Esum Mathias Eyong a,1, Sophie Jose Molua Etutu a, Fru-Cho Jerome a, Raymond Babila Nyasa a, Tebit Emmanuel Kventi b, Marcel N. Moyeh c

a Department of Microbiology and Parasitology, Faculty of Science, University of Buea, P.O. Box 63, Buea, South West Region, Cameroon
b Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Buea, P.O. Box 63, Buea, South West Region, Cameroon
c Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P.O. Box 63, Buea, South West Region of Cameroon

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ABSTRACT

Objective: Plasmodium falciparum produces histidine-rich protein 2/3 (Pfhrp2/3) genes that accumulate to high levels in the bloodstream and serve as a diagnostic and prognostic marker for falciparum malaria. Pfhrp2/3 gene deletions may lead to false-negative rapid diagnostic test (RDT) results. We aimed to determine the prevalence of Pfhrp2/3 gene deletions in P. falciparum isolates and the implications for RDT use in the Mount Cameroon region.

Methods: A cross-sectional hospital-based study with malaria diagnosis performed using microscopy, RDT and nested polymerase chain reaction (nPCR). In total, 324 P. falciparum microscopy positive individuals were enrolled and their samples confirmed positive for P. falciparum using 18SrRNA PCR. Samples that gave false-negative RDT results were analyzed to detect pfhrp2/3 exon 2 deletions.

Results: Of 324 positive microscopic and nPCR samples, 16 gave RDT false-negative results. Among the 324 P. falciparum positive isolates, exon 2 deletions were observed in 2.2% (7 of 324); 3 were negative for pfhrp2 gene, 2 for pfhrp3, and 2 for both pfhrp2 and pfhrp3 (double deletions).

Conclusion: P. falciparum isolates with pfhrp2/3 gene deletion were present in the parasite populations and may contribute to the RDT false-negative results in the Mount Cameroon region.

1.1. Introduction

Malaria is an infectious disease with an estimated 229 million cases globally; it was responsible for 409,000 deaths in 2019 (World Health Organization, 2020). In the same year, Plasmodium falciparum malaria was responsible for nearly all malaria cases and fatalities in Sub-Saharan Africa and 94% of all malaria cases and deaths worldwide (World Health Organization, 2020). Malaria caused by the falciparum parasite continues to have a detrimental impact on human life and fragile economies.

Malaria remains prevalent in Cameroon, with an estimated 71% of the population living in high transmission areas (World Health Organization, 2011), and is the leading cause of morbidity and mortality amongst the most vulnerable groups, including children under the age of 5, pregnant women and the poor. Effective malaria therapy necessitates accurate laboratory diagnosis, which is still a key component of global malaria control efforts (Ali et al., 2016).

Malaria rapid diagnostic tests (RDTs) based on histidine-rich protein-2 (hrp2) have improved malaria case management and surveillance, especially in Africa, where P. falciparum is most common. The emergence of gene deletion on P. falciparum histidine-rich proteins 2 (pfhrp2) and 3 (pfhrp3) has jeopardized the utility of these RDTs. Any pfhrp2 or pfhrp3 gene deletion may impact the performance of Pfhrp2-based RDTs, leading to incorrect patient diagnosis and therapy. For reliable investigation, confirmation and reporting of pfhrp2 and pfhrp3 gene deletion, standard and recommended methods must be used. Agaba et al. (2019) and Kojom and Singh (2020) conducted systematic reviews and found unambiguous evidence of pfhrp2 and pfhrp3 gene deletion in Africa, where P. falciparum is the most common pathogen, and HRP2-based RDTs are the most utilized for malaria diagnosis. There is currently no information on the deletion of the pfhrp2 and pfhrp3 genes in Cameroon.

2.1. Materials and methods

2.1.1. Study design

We conducted a hospital-based cross-sectional study from August 2020 to July 2021 involving febrile patients seeking malaria diagnosis in the regional hospitals in Buea and Limbe in the Mount Cameroon Region.
2.1.2. Study area

Several studies in Africa have reported the presence of pfhrp2/3 gene deletions. Our study was carried out in the Mount Cameroon region, situated in Fako Division (4°10’00”N, 9° 10’00”E) and covering an area of 2093 km² with an elevation ranging from 0 to 4095 m above sea level (Fig. 1). The region has an estimated population of 534,854 and the major towns include Buea, Idenau, Limbe, Mutengene, Muyuka and Tiko. The climate is generally hot and dry, except for Buea, which has a humid climate due to its location on the slopes of Mount Cameroon. Fako Division experiences two seasons, rainy and dry. The rainy season is usually between March and October, and the dry season is between November and February.

2.1.3. Study participants

Our study involved patients coming for consultation in the Out-Patient Department or Emergency Unit of the regional hospitals who consented to participate in the study. They were febrile patients of both sexes and all ages residing in the study area who presented with malaria symptoms but were not on any antimalarial medication.

2.1.4. Sampling technique

A simple random sampling technique was used to enrol participants with a sample size calculated using the formula described by Swinscow (2002):

\[ n = \frac{Z^2 \times p \times (1 - p)}{\epsilon^2} \]

where

- \( n \) = sample size
- \( p \) = prevalence of deletion from previous study in Nigeria = 17% (Funwei et al., 2019)
- \( Z = 95\% CI = 1.96 \)
- \( c = error = 0.05 \)

\[ n = \frac{1.96^2 \times 0.17 \times (1 - 0.17)}{0.05^2} \]

\[ n = 217 \]

Hence, we required approximately 300 participants to adjust for dropouts.

2.1.5. Data collection

A structured questionnaire was used to gather study participants’ knowledge of malaria and preventive methods. Participants’ axillary temperature was measured using an electronic thermometer; fever was defined as ≥37.6°C (Sumbele et al., 2016).

2.1.6. Sample collection

Venous blood (2–5 mL) was collected from each participant and dispensed into ethylenediaminetetraacetic acid tubes using antiseptic techniques. The uncoagulated blood was used to perform the malaria diagnostic tests (microscopy, RDT and polymerase chain reaction [PCR]). Thick/thin blood smear and RDT for malaria were conducted for all participants. A few drops of blood were spotted on labelled filter paper (Whatman No.3, Sigma-Aldrich, Germany) and allowed to air dry. The papers with dried blood spots were individually stored in plastic bags with a desiccant at room temperature for subsequent DNA extraction.

2.1.7. Sample analysis

2.1.7.1. Detection of malaria parasite by Giemsa microscopy. The blood films were prepared, air-dried and stained with freshly prepared 10%...
Giemsa solution for 25–30 min according to the standard World Health Organization (WHO) Methods Manual procedure (World Health Organization, 2015) and read separately by two expert microscopists. A third microscopist resolved any discrepancy between readings. Detection of malaria parasites and estimation of the parasite density by light microscopy was performed as described in the WHO Methods Manual (World Health Organization, 2015).

2.1.7.2. Diagnosis of malaria parasite by rapid diagnostic test. Two commercially available RDT kits, CareStart Malaria P/PAN (HRP2/pLDH) (ACCESSBIO, New Jersey, USA) Ag Combo RDT and SD BIOLINE Malaria Ag P.f (Standard Diagnostics Inc, Korea) RDT, were used to detect malaria parasites, according to the manufacturer’s instructions.

The CareStart Malaria P/PAN (HRP2/pLDH) Ag Combo RDT is a chromatographic test for the rapid qualitative detection of malaria hrp2 and pLDH (plasmidium lactate dehydrogenase) of P. falciparum, P. vivax, P. ovale and P. malariae in human whole blood. The RDT membrane strip is pre-coated with two monoclonal antibodies as two independent lines across the test strip. One line (test line 2) is pan-specific to pLDH of the Plasmodium species and the other line (test line 1) contains a monoclonal antibody specific to P. falciparum HRP2. Antibodies absorbed in gold particles are impregnated on the conjugate pad. The test had a sensitivity of 98% (95% CI 97.05%–100%) and specificity of 97.5% (95% CI 94.64%–99.36%).

The SD BIOLINE Malaria Ag P.f RDT is a fast chromatographic test for the qualitative detection of the hrp2 antigen of malaria P. falciparum in human whole blood. On the test line region, a membrane strip is pre-coated with mouse monoclonal antibodies specific to HRP2 of P. falciparum. The malaria P. falciparum antigen in the specimen reacts with the mouse monoclonal antibodies specific to HRP2 of P. falciparum colloidal gold conjugate. They chromatographically migrate through the membrane to the test region, where they form a visible line as an antibody-antigen-antibody gold particle complex with a high degree of sensitivity and specificity. The test had a sensitivity of 99.7% (95% CI 98.5%–100%) and specificity of 99.5% (95% CI 97.2%–99.9%).

2.1.7.3. Detection of malaria parasite by PCR.

2.1.7.3.1. Parasite DNA extraction. The DNA template for the nested PCR (nPCR) assay was extracted from dried blood on filter paper using the Chelex 100 method, as previously described (Musapa et al., 2013). Then, 120 μL of the total genomic DNA was carefully transferred into sterile Eppendorf tubes and stored at -20°C for future use.

2.1.7.3.2 P. falciparum identification by nested PCR. Detection of malaria parasite DNA was based on nPCR amplification of the 18S rRNA gene in a reaction that used 2 μL of the extracted DNA, 7.5 μL of GoTaq polymerase and master mixes (Promega, USA), 0.5 μL each of upstream and downstream primers, and 4.5 μL of nuclelease-free water in a total reaction volume of 15 μL. The first PCR included genus-specific primers, while the second nPCR run included P. falciparum-specific primers (species-specific primers), as previously described (Abdallah et al., 2015), using the primary amplicons as the DNA template (Table 1). nPCR was repeated for all negative results. For discordant results, amplification was repeated a third time, with the final result being two consistent results. The thermocycling conditions were as shown in Table 2.

2.1.7.3.3 nPCR amplifications of the pfhrp2 and pfhrp3 genes. Positive P. falciparum samples that were RDT-negative were used for further amplification of the exon 2 of pfhrp2 and pfhrp3 genes to detect the presence or absence of these two genes. The primary PCR amplification of pfhrp2 and pfhrp3 genes was performed using the primers and reaction conditions described in Tables 1 and 3. These amplifications were performed in a total volume of 15 μL consisting of 7.5 μL of One Taq 2X master mix with standard buffer, 0.5 μL each of outer and nested primers, 2 μL of DNA template and 4.5 μL of nuclelease-free water. The nPCR was performed in the same manner with the amplicons from the primary reaction being used as the DNA template. nPCR was repeated for all negative results. In the instance of contradictory results, the amplification was repeated a third time, with the final result being two consistent results.

2.1.7.4. Confirmation of gene deletion. To verify the presence of parasites in the pfhrp2-negative samples, microscopy-positive samples with positive results for 18S rRNA but negative results for pfhrp2/3 were considered pfhrp2/3-deleted after excluding low parasitaemia to avoid incorrect deletion calls.

2.1.7.5. Agarose gel electrophoresis. A 2% (w/v) agarose gel was used to confirm the presence of bands. The amplified PCR products (amplicons) were detected by running 1 μL of the PCR mixture on the agarose gel, stained with 0.5 μg/mL ethidium bromide solution. The samples were run using a Powerpack (Biorad, CA, USA) at 99 volts for 30 min alongside a 100 bp DNA ladder and a negative control (autoclaved distilled water), followed by separation. The bands were visualized under a UV transilluminator (Bio-Rad).

Table 1
Prime sequences for amplification of 18S rRNA gene, pfhrp2 and pfhrp3 genes in malaria parasites

| Species          | Primer          | Sequence (5’-3’)          | Expected fragment length in Base pairs (bp) |
|------------------|-----------------|---------------------------|---------------------------------------------|
| Plasmodium spp.  | rPLU5           | CGTGTGTTGGTTAAAACCTC      | 900                                         |
|                  | rPLU6           | TTTAATTTTTGAGTTAAAAAGG    | 205                                         |
| P. falciparum    | rFA1            | TTTAAGCTATTGTTAATAAAGG    | 205                                         |
|                  | rFA2            | TTTAAGCTATTGTTAATAAAGG    | 205                                         |
| pfhrp2           | Outer           | ATTATTCAACAGAACATCAGACAC  | 600 – 1000                                  |
|                  | Nested          | ATTTATTCAACAGAACATCAGACAC | 600 – 1000                                  |
| pfhrp3           | Outer           | AACAGAAAAAATTAATACAGAAAG | 600 – 950                                   |
|                  | Nested          | TCTGAATTCATCAACAGAAAG     | 600 – 950                                   |

Table 2
Thermocycling conditions for amplification of Plasmodium falciparum

| Steps                | Temperature | Time |
|----------------------|-------------|------|
| Pre-denaturation      | 94°C        | 3 minutes |
| Denaturation          | 94°C        | 1 minute |
| Annealing             | 55°C        | 1 minute |
| Extension             | 68°C        | 1 minute |
| Number of Cycles      | 25          | 30   |
| Final extension       | 68°C        | 5 minutes |
| Hold                  | 4°C         |      |
2.1.7.6. Data analysis. Data collected were entered into Census and Survey Processing System (CassPro 7.6 software) and analyzed using IBM® SPSS® Statistics version 20 (IBM, USA) for Microsoft Windows. Pearson’s chi-square test was performed. Data were double-checked to detect missing data or errors. The threshold for statistical significance was set at P<0.05.

3.1. Results

3.1.1. Sociodemographic and clinical characteristics of study participants

A total of 324 participants who were positive by microscopy for *P. falciparum* malaria were enrolled in the study: 201 (62.0%) were women and 123 (37.8%) men, 36% (119 of 324) were aged <20 years, 47.5% (154 of 324) had a secondary school level education, and 50.6% (164 of 324) were single. Table 4 provides the sociodemographic and clinical characteristics of the participants.

The mean body temperature of the participants was 37.9°C ± 0.49 (Table 4). All participants had a past malaria infection with a history of fever: 47.2% (153 of 324) had been infected >3 times between birth and when they were enrolled in this study and 58.3% (189 of 324) had received malaria treatments from health facilities.

3.1.3. Detection of *P. falciparum* malaria

Of the 324 microscopy positive samples, 308 (95.1%) were RDT-positive and 16 (4.9%) were RDT-negative. nPCR confirmed all samples were positive for *Plasmodium* infection. *P. falciparum* was the only species detected in the positive samples by both microscopy and PCR. Table 5 shows the results of malaria detection using microscopy, RDT and PCR.

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**Table 3**
Thermocycling conditions for amplification of Pfhrp2 and Pfhrp3 Genes

| Species | Initial Denaturation | Denaturation | Annealing | Extension | Cycles | Final extension | Hold |
|---------|----------------------|--------------|-----------|-----------|--------|-----------------|------|
| Pfhrp2  | 94°C for 5 minutes   | 94°C for 30 seconds | 59°C for 1 minute | 68°C for 1 minute | 40     | 68°C for 3 minutes | 4°C  |
| Nested  | 94°C for 5 minutes   | 94°C for 30 seconds | 59°C for 1 minute | 68°C for 1 minute | 40     | 68°C for 3 minutes | 4°C  |

**Table 4**
Socio-demographic and Clinical characteristics of study participants

| Characteristics                          | Variable                          | Number       | Percentage   |
|------------------------------------------|-----------------------------------|--------------|--------------|
| Gender                                   | Male:Female:Total                  | 123:201:324  | 38.062.0100  |
| Age Group (Years)                        | < 20:20-29:30:30-49:50:Total       | 119:84:42:95:03:24 | 36.725.913.09.015.4100 |
| Mean ± SD age (Years)                    | 27.45 ± 19.41-99                   |              |              |
| Educational Level                        | Non-formal education:Primary:Secondary:Tertiary:Vocational:training:Total | 94:115:41:01:19:32:4 | 2.812.747.531.25.9100 |
| Marital Status                           | Single:Married:Widowed:Total       | 16:41:91:11:32:4 | 50.646.03.4100 |
| Temperature                              | 37.5-38.3:38.4-39.4:39.5:Total     | 276:399:32:4 | 85.212.02.8100 |
| Mean Temp.                               | 37.9 ± 0.49                       |              |              |
| Number of times infected                 | 12:3:Total                        | 36:77:51:53:2:4 | 11.123.817.947.2100 |
| Parasitemia (T/µL)                       | < 1:00:0:01:00:49:99:99:99:99:0:0:0:Total | 74:117:108:53:2:4 | 22.842.333.31.5100 |
| Mean density ± SD Min – Max              | 11 825.99 ± 33 033.24180 – 344 727 |              |              |

**Table 5**
Microscopy, RDT, and nPCR results for detection of *P. falciparum*

| Variable | Examined | Positive n (%) | Negative n (%) |
|----------|----------|----------------|----------------|
| Microscopy | 324      | 324 (100)      | 0 (0.0)        |
| RDT 1 (CareStart™ Malaria PI/PAN) | 324 | 308 (95.1) | 16 (4.9) |
| RDT 2 (SD BIOLINE Malaria Ag P.f) | 324 | 308 (95.1) | 16 (4.9) |
| nPCR     | 324      | 324 (100)      | 0 (0.0)        |

Abbreviations: RDT, rapid diagnostic test; nPCR, nested polymerase chain reaction

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Fig. 2. Nested PCR of samples on gel representing *pfhrp2* genes M= molecular weight marker; 3 is the negative control; 5 is a negative sample; 1, 2, 4, are positive samples

Fig. 3. Nested PCR of samples on gel representing *pfhrp3* genes
M= molecular weight marker; 3 is the negative control; 5 is a negative sample; 1, 2, 4 and 6 are positive samples
Table 6
Diagnostic profile and parasite density of the microscopy positive/RDT negative isolates

| SN | Sample ID | Microscopy | Results |
|----|-----------|------------|---------|
|    |           |            | RDT1   | RDT2   | nPCR  | pfhrp2 | pfhrp3 | Parasites/μL |
| 1  | LO02      | +          | -      | -      | +     | +      | +      | 3,200     |
| 2  | LO52      | +          | -      | -      | +     | +      | +      | 3,800     |
| 3  | LO69      | +          | -      | -      | +     | +      | +      | 240       |
| 4  | LO77      | +          | -      | -      | +     | +      | +      | 400       |
| 5  | LO94      | +          | -      | -      | +     | +      | +      | 880       |
| 6  | LO95      | +          | -      | -      | +     | +      | +      | 240       |
| 7  | LI02      | +          | -      | -      | +     | +      | +      | 3,200     |
| 8  | LI04      | +          | -      | -      | +     | +      | +      | 480       |
| 9  | LI28      | +          | -      | -      | +     | +      | +      | 780       |
| 10 | B001      | -          | -      | -      | -     | +      | +      | 3,000     |
| 11 | B004      | -          | -      | -      | -     | +      | +      | 7000      |
| 12 | B005      | -          | -      | -      | -     | +      | +      | 16,000    |
| 13 | B056      | -          | -      | -      | -     | +      | +      | 8,000     |
| 14 | B114      | -          | -      | -      | -     | +      | +      | 180       |
| 15 | B123      | +          | -      | -      | -     | +      | +      | 1,592     |
| 16 | B124      | +          | -      | -      | -     | +      | +      | 6,766     |

Table 7
Prevalence of P. falciparum hrp2/hrp3 deletions in the false-negative isolates (n=16)

| Variable | Number | Percentage (95% CI) |
|----------|--------|---------------------|
| pfhrp2   |        |                     |
| Positive | 11     | 68.8 (43.8-87.5)    |
| Negative | 5      | 31.3 (12.5-56.3)    |
| pfhrp3   |        |                     |
| Positive | 12     | 75.0 (50.0-93.8)    |
| Negative | 4      | 25.0 (6.3-50.0)     |

3.1.4. Amplification of pfhrp2 and pfhrp3 genes
Amplification of pfhrp2 and pfhrp3 genes was done on the 16 samples that were RDT negative but PCR positive. Of these samples, 2 were negative for both pfhrp2 and pfhrp3 genes (double deletions), 3 negative for pfhrp2 genes only, and 2 negative for pfhrp3 genes only. Figures 2 and 3 show the PCR gel banding patterns of the pfhrp2 and pfhrp3 genes.

Of the 16 samples, 11 (68.8%) were positive for pfhrp2 genes and 12 (75.0%) were positive for pfhrp3 genes. Nine had coexisting positive results for both pfhrp2 and pfhrp3 genes, 2 were positive only for pfhrp2 genes and 3 were positive only for pfhrp3 genes, as shown in Table 6.

Out of the 7 samples with pfhrp2/3 gene deletions, PCR assay identified 3 (42.9%) as deleted for pfhrp2 genes only, 2 (28.6%) as deleted for pfhrp3 genes only, and 2 (28.6%) deleted for both pfhrp2 and pfhrp3 genes (fig. 4A).

3.1.5. Prevalence of pfhrp2 and pfhrp3 gene deletions in the total isolates and the false-negative RDT samples
Out of the 324 samples, the prevalence of pfhrp2/3 gene deletion was 2.2% (7 of 324) (Fig. 4B). The prevalence of pfhrp2 gene deletion was 1.5% (5 of 324) and 1.2% (4 of 324) for pfhrp3 gene deletion, with 2 of these samples having deletions for both pfhrp2 and pfhrp3 genes (0.6% prevalence). Amplification of the Plasmodium 18SrRNA identified 16 samples to have false-negative RDT results. Of these 16 samples, 43.8% (7 of 16) had pfhrp2 gene deletion (Fig. 4C), 31.3% (5 of 16) had pfhrp2 gene deletion and 25.0% (4 of 16) had pfhrp3 gene deletion (Table 7). A total of 2 isolates were negative for both pfhrp2 and pfhrp3 giving a prevalence of 12.5% for double deletions in the false-negative RDT isolates.

4.1. Discussion
In our study, 7 of the 324 participants had pfhrp2/3 gene deletion giving a prevalence of 2.2%, similar to a study carried out in Mali which found a 2.0% prevalence. However, the prevalence in this study is far less than the 62.0% prevalence observed in Eritrea (Berhane et al., 2018). In the absence of the pfhrp2 protein, most of the antibodies used in RDTs to detect pfhrp2 also detect pfhrp3 due to structural homology (Gendrot et al., 2019).

The prevalence of pfhrp2 exon 2 deletions was 1.5% (5 of 324) in this study, which is comparable to previous studies in Senegal (2.4%), Mozambique (1.45%) and Ethiopia (4.8%) (Wurtz et al., 2013; Gupta et al., 2017; Girma et al., 2018), and lower than findings reported in Ghana (36.2%, Amoah et al., 2016), DR Congo (6.4%, Parr et al., 2017), Kenya (9% Beshir et al., 2017), Eritrea (9.7%, Menegon et al., 2017), Rwanda (23%, Kozycki et al., 2017), Sudan (60%, Hamid et al., 2017), Eritrea (62%, Berhane et al., 2018), Zambia (37.5%, Kobayashi et al., 2019), Nigeria (17%, Funwei et al., 2019) and Ethiopia (17.9%, Alemayehu et al., 2021).

Similarly, the prevalence of pfhrp3 exon 2 deletion (1.2%; 4 of 324) was comparable to studies in Kenya which found a prevalence of 1.1% (Beshir et al., 2017) but lower than the prevalence of deletion reported in Honduras, 44.1% (Abdallah et al., 2015), Eritrea 100% (Berhane et al., 2018) and Ethiopia 9.2% (Alemayehu et al., 2021). Variations in transmission intensity, geographical location, sample size, and laboratory methods used to analyze pfhrp2/3 genes deletions could explain these discrepancies.

In the present study, 47.8% (7 of 16) of PfHRP2 RDT negative samples lacked exon 2 of pfhrp2/3, the main amino acid coding region of the pfhrp2 and pfhrp3 gene. Of these PCR-negative pfhrp2/3 exon 2 samples, all were microscopy positive. The pfhrp2/3 gene deletions observed in this study would undoubtedly impact the diagnostic performance of PfHRP2 RDTs. The WHO’s threshold level for pfhrp2/3 deletions causing false-negative RDT results is much lower at 5% (World Health Organization, 2018). Our study also revealed that 68.75% (11 of 16) of false-negative PfHRP2 RDT results were pfhrp2 exon 2 positive. The cause of these false-negative PfHRP2 RDT results could, as reported in previous studies, be due to the absence of PfHRP2 antigen as a result of host immune response in high transmission areas (Ho et al., 2014), variation in the amino acid sequence expressing reduced levels of the target antigens (Cheng et al., 2014), or the genetic variation of the pfhrp2 gene leading to a modified protein PfHRP2 with a new epitope that is not recognized by RDT antibodies (Gendrot et al., 2018).

The proportion of isolates with pfhrp2 gene deletions among the false-negative cases using PfHRP2-based RDT was 31.3% (5 of 16). A similar result (23%) was reported in Ghana (Amoah et al., 2016). Conversely, only 2.4%, 10.6% and 9.9% of false-negative PfHRP2-RDT results involved parasites with pfhrp2 gene deletions were reported in Senegal (Wurtz et al., 2013), Nigeria (Funwei et al., 2019) and DR Congo (Munyeku et al., 2021), respectively. Similarly, the prevalence of isolates...
with pfhrp3 gene deletions among the false-negative cases using PfHRP2-based RDT was 25.0% (4 of 16), differing from the 85.8% prevalence (6 of 7) recorded in Senegal (Wutz et al., 2013).

Genetic alterations in parasites, such as pfhrp2/3 deletion, are unlikely to be the only source of RDT false negatives. Some of the RDT false-negatives samples were found to be positive using nPCR and there were no identifiable deletions in the pfhrp2/3 genes. These findings could be attributed to issues with the RDT employed or operator error in completing the tests and/or interpreting the results, which could lead to false negatives, as documented in prior research (Berzosa et al., 2020).

Partially deleted pfhrp2 and pfhrp3 genes, prozone effects due to abundant antigen, sequence variability of pfhrp2 and pfhrp3 genes, and circulating antibodies to HRP2 have all been observed to interfere with HRP2 RDT detection (Gamboa et al., 2010; Lee et al., 2006; Ho et al., 2014). Although the primers employed in this investigation only amplified exon 2 of the pfhrp2 gene and exon 2 of the pfhrp3 gene, the pfhrp2 and pfhrp3 genes are known to contain chromosomal breaking sites outside of exon 2 (Cheng et al., 2014). In individuals with hyperparasitaemia, HRP2-based RDTs have been reported to show prozone-like effects. Although the mechanism of prozone-like effects for antigen detection assays is unknown, one likely explanation is that the amount of antigen detected exceeds the dye-labeled antibodies' binding capability. Unlabeled target antigen reaches the test strip in this case, saturating the binding capacity of the test strip's capture antibodies. As a result, dye-labeled antibodies that capture antigen may fail to bind to the test strip and generate a visible band (Luchavez et al., 2011). However, the prozone impact has only been linked to false-negative HRP2 test lines in samples with ≥288,000 parasites/μL (Gillet et al., 2011). Therefore, based on our parasite density data, it is unlikely to have been a significant cause of false-negative RDTs in this study.

This study mentions the role of an intact pfhrp3 gene in PfHRP2 RDT-positive results. Although only three of the pfhrp2 exon 2 PCR positive samples lacked pfhrp2 exon 2, these three samples were confirmed positive by PCR and microscopy in the absence of the pfhrp2 gene, suggesting that pfhrp3 exon 2 may have contributed. This finding aligns with previous studies (Amoah et al., 2016; Beshir et al., 2017; Alemayehu et al., 2021) which showed that structural similarities between epitopes of PfHRP2 and PfHRP3 antigens allow PfHRP2 monoclonal antibodies to cross-react with PfHRP3 (Lee et al., 2012). Indeed, the true prevalence of pfhrp2 gene deletion in our study may have been underestimated by PfHRP2 RDT positive test results with intact pfhrp3 exon 2.

Our findings demonstrate that pfhrp2-negative parasites are more common in our study site than pfhrp3-negative parasites, which has also been seen in Suriname (Akinyi Okoth et al., 2015). In Colombia, Peru, and Honduras (Gamboa et al., 2010; Akinyi et al., 2013; Abdallah et al., 2015), pfhrp3-negative parasites outnumber pfhrp2-negative parasites.

5.1. Conclusion

Our study revealed that P. falciparum isolates with pfhrp2 and pfhrp3 gene deletions are present in the parasite populations in the Mount Cameroon region. False-negative RDT results may lead to misdiagno-
sis of patients with malaria infections where the diagnosis is based on the use of PfHRP2-based RDTs only.

It is essential to have good diagnostic tools on the front line to control malaria. Therefore, there is a need for more extensive studies involving samples collected from different geographical settings across Cameroon to estimate the true prevalence of pfhrp2/3 deleted parasites and their impact on malaria diagnosis in Cameroon.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors’ contributions

EME: Methodology, supervision, formal analysis, writing – original draft of the manuscript. SJME: Conceptualization, methodology, data collection, review and editing. FCJ: Co-supervision, review and editing. RBN: Co-supervision, review and editing. TEK: Data collection, editing. MNM: Resources, review. All authors discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials supporting the results of the manuscript are available from eyong.mathias@ubuea.cm / mathias_mesum@yahoo.fr upon request.

Consent for publication

All authors have seen and approved the final version of the manuscript for publication in the International Journal of Infectious Diseases.

Ethics approval and consent to participate

Ethical clearance for this study was obtained from the Institutional Review Board of the Faculty of Health Sciences, the University of Buea, Cameroon (Ref: 2021/1255-11/UB/SG/IRB/FHS). Authorization was obtained from the Ministry of Public Health, Regional Delegation for the Southwest Region (Ref: R11/MINSANTE-SWR/RDPH/PS/240/986). Further administrative authorization was obtained from the Medical Directors of the Regional Hospital Buea Annex and the Regional Hospital Limbe. Written informed consent was obtained from all participants ≥18 years of age, and parents or legal guardians of children <18 years gave written informed consent on behalf of their children before their inclusion in the study.

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E.M. Ryong, S.J.M. Etatu, F.-C. Jerome et al.

UID Regions 3 (2022) 300–307

307