Molecular Surveillance of the *pfhrp2* and *pfhrp3* Genes in *P. falciparum*

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Introduction: *Plasmodium falciparum* is the species responsible for most malaria deaths worldwide, especially in sub-Saharan Africa, and it causes the most severe cases of malaria. Because of these traits, this species can lead to serious complications if not diagnosed quickly and accurately.

Therefore, a prompt and accurate diagnosis is essential for patients suspected of having malaria. With the worldwide emergence of deletions in the *pfhrp2* and *pfhrp3* genes, the aim of this research is to determine whether the prevalence of these deletions, which lead to false-negative rapid diagnostic test (RDT) results, has reached a level where changing test use is necessary. Given this context, due to the limited data available in Brazil and the differences observed among previously published studies, there is no expected prevalence rate for these deletions in the national setting.

Objective: To investigate deletions in the *pfhrp2* and *pfhrp3* genes of *P. falciparum* samples from the Brazilian Amazon and Africa.

Methodology/Experimental Design: Our study is a descriptive, non-analytical investigation with both retrospective and prospective components. The methodology involves determining the frequency of gene deletions and mutations among patients seeking malaria diagnosis at the Center for Research, Diagnosis, and Training in Malaria (CPDMal).

To achieve this, nested PCR targeting exons 1 and 2 of *pfhrp2* and *pfhrp3*, and single PCR targeting exon 2 of both genes, was performed. Amplicons were analyzed for mutations using Sanger sequencing.

Results: To date, 20 *P. falciparum* samples have been amplified across the exons 1-2 of the *pfhrp2* gene, and five samples for exons 1-2 of the *pfhrp3* gene. Ten samples were amplified for exon 2 of the *pfhrp3* gene and 3 for the *pfhrp2* gene.

All these samples were successfully sequenced, and none showed deletions in these genes.

Conclusion: In this small initial sample, the HRP2 and HRP3-based rapid diagnostic tests performed effectively. Additional samples are being analyzed to draw robust conclusions.

Source of funding: CNPq; PNCM/SVSA/MS; FAPERJ; Fiocruz