

## Review Article

# Methodological discrepancies of Reports on *Plasmodium Falciparum* Histidine Rich Protein 2/3 Gene Deletions among African Countries Between January 2010 And December 2021: A Systematic Review

Keywords: *Plasmodium falciparum*, gene, deletion, histidine.

### ABSTRACT

**Background** *P. falciparum* histidine rich protein 2/3 gene deletions (Pfhrp2/3gd) threaten usefulness of mRDT in diagnosing malaria cases. This review was performed to assess the methods used to report findings on those deletions between January 2010 and December 2021.

**Methods:** Present search was performed using Medline, Google Scholar and PubMed.

**Results:** Out of 94 articles identified, 19% (n=18) were included. Reviewed articles varied in methods employed to report those deletions.

**Conclusion:** Methodological approaches for the Reports between January 2010 and December 2021 on Pfhrp2/3gd are varying and this implies that the findings may not be generalized.

### INTRODUCTION

Malaria is a disease of public health importance and affects many people in Africa (1). In 2015, there were around 429,000 deaths affecting mainly children under five years in African countries (2). The disease causes mortality and morbidity in pregnant women (3,4). In Tanzania, strategies to control malaria include distribution of insecticide treated mosquito bed nets to under-fives, indoor residual spray and intermittent preventive treatment during pregnancy with Sulfadoxine pyrimethamine (IPTp-SP) (5) that aims at reducing poor pregnancy outcomes (6), and subsequently reduce incidences of congenital malaria in newborns (7,8).

So far, malaria rapid diagnostic tests (mRDTs) are widely used in African countries to diagnose malaria cases such that their accuracy is a prerequisite to successful management of malaria cases (9). However their usefulness has been threatened by reports on *P. falciparum* histidine rich protein 2/3 gene deletions (pfhrp2/3gd) (10–16) which may lead to false negative results paving way to poor management of malaria cases.

This review was performed to assess variation of methodological approaches of studies on Pfhrp2/3gd with a focus on their study design, sample size, study areas, merozoite surface protein I/II polymerase chain reaction reports, assessment on flanking genes and type of research participants.

### METHODS

#### Searching techniques

Abstracts and paper titles were employed in this review. English language was employed in systematic search. Literature search was performed through Google Scholar, PubMed and MEDLINE. Search items used were 'deletion', '*Plasmodium falciparum*', 'Pfhrp2', 'Pfhrp3', 'Malaria Rapid diagnostic tests', 'Africa'.

#### Review aims

Present review aimed at assessing consistency in methodological approaches for reports between January 2010 and December 2021 on Pfhrp2/3gd.

### Inclusion criteria for articles

Only findings which met inclusion criteria were reviewed. Inclusion criteria for articles were (a) Primary data published between January 2010 and December 2021 (b) Area of study found in Africa (c) Reporting on Pfhrp2/3gd.

### RESULTS

Tentative online search resulted into 94 articles. Out of 94 articles, 19.2% (n=18) met criteria for review while 80.8% (n=76) did not meet criteria for review and these included review articles, articles published outside selected time frame, studies conducted in non-African countries and reports which did not aim at determining those deletions. Detailed information on methods used for reviewed articles are depicted on Table 1.

**Table 1: Profile of methodological approaches of Reviewed reports on Pfhrp2/3gd**

African Country	First author	Study design and type of participants	SZ	Pfhrp2/3gd	FG	LM	SGD	Year of publication
Ethiopia (18)	Alemayehu	CSS	218	BR	R	BM1/2N	17.9	2021
Uganda (19)	Sam	CSA	1493	BR	NR	BM1/2N	4.2	2021
Tanzania (17)	Bakari	CSA	7543	BR	NR	BM1/2N	0	2020
Mali (20)	Koita	CSA	480	O2R	NR	MSP2 PCR not reported	2%	2012
Kenya (21) (22)	Beshir	CSA	131	BR	NR	BM1/2R	10a	2018
	Nderu	CSS	400	BR	NR	BM1/2N	0	2017
DRC (11)	Parr	CSA	2752	BR	NR	BM1/2N	6.4%	2017
Eritrea (23)(24)	Berhane	CSS	50	BR	R	BM1/2R	6.2%	2017
	Menegon	CSA	144	BR	NR	BM1/2N	9.7	2018
Rwanda (25)	Kozycki	CSS	3291	O2R	NR	BM1/2N	23a	2017
Mozambique (26)	Gupta	CSS	1162	BR	NR	BM1/2N	1.45a	2017
Senegal (27)	Wurtz	CSS	112	BR	NR	BM1/2N	2.4	2013
Ghana (28)	Amoah	CSA	94	O2R	NR	BM1/2N	36.2	2016
Nigeria (29)	Funwei	PC	309	BR	NR	BM1/2R	17a	2019

Zambia(30)	Kobayashi	CSA	28	BR	NR	BM1/2N	10.7	2019
Angola (16)	Plucinski	CSA	466	BR	NR	BM1/2N	0.4	2019
Ethiopia (31)	Girma	CSA	562	O2R	NR	BM1/2N	4.8a	2019
Ethiopia (32)	Golassa	CSS	189	BR	R	BM1/2R	22	2020

Key: CSS=Cross sectional symptomatic, CSA= Cross sectional Asymptomatic, PC=Prospective cohort, SZ= sample size, FG= Flanking genes, LM =Laboratory method, SGD=Status of reported deletions, O2R=Only Pfhrr2 gene reported, BR=Both genes reported, NR= Flanking genes not reported, R= Flanking genes reported, BM1/2N=Both MSP1 and MSP2 PCR results not available, BM1/2R= Both MSP1 and MSP2 PCR results available

## DISCUSSION

Methodological approaches employed to report Pfhrr2/3/gd between January 2010 and December 2021 in African countries were assessed in present review. Generally, the review shows methodological discrepancies as shown in Table 1.

In the year 2018, The World health organization launched a standard guideline for the estimation of Pfhrr2/3gd. In this review, out of 18 reviewed reports 44.4% (n= 8) were reported after the publication of the standard guideline (33) while 55.6% (n=10) were published prior to the guideline. In this review, 5.6% (n=1) of reviewed reports, study designs were prospective cohort study while remaining studies 94.4% (n=17) were cross sectional designs. However, in order to estimate the prevalence of Pfhrr2/3gd in *Plasmodium falciparum* populations, the recommended study design is cross sectional with symptomatic research participants since the parasitemia levels are likely to be higher in symptomatic individuals than asymptomatic individuals(33).

In this review, sample size for study participants ranged from lowest 28 research participants(30) to highest 7543 research participants (17). The WHO guideline recommended at least 370 individuals enrolled from 10 randomly selected facilities per survey domain or region. In this regard the domains need to include a combination of low, moderate and high malaria transmission areas. In this review, none of the studies considered a combination of low, moderate and high malaria transmission areas such that estimation of the prevalence of Pfhrr2/3gd in *Plasmodium falciparum* populations was not in agreement with the standard WHO guideline. Studies on Pfhrr2/3/gd which aim at informing on the prevalence of Pfhrr2/3gd in the *Plasmodium falciparum* populations according to the standard guideline should report both Pfhrr2 and Pfhrr3 genes. However, in the reviewed reports, 22.2% (n=4) did not assess both deletions. Furthermore, out of total 18 reviewed reports, flanking genes were assessed in 16.7% (n=3) of the reviewed articles while the rest of the reviewed articles did not report on the flanking genes as shown on Table 1. Such a methodological approach may lead to improper estimation of the Pfhrr2/3gd in *Plasmodium falciparum* populations. Furthermore, in the present review, 77.8% (n=14) of the reviewed articles did not report both merozoite surface protein I (MSPI) and merozoite surface protein II (MSPII) polymerase chain reaction findings contrary to the recommended WHO guidelines which recommends both MSP1 and MSP2 PCR tests to be performed (33).

Findings of this review imply that researchers employ various methods in reporting Pfhrr2/3gd in African countries and that may have implications on decisions and policy.

## CONCLUSION

Reports on *Plasmodium falciparum* histidine rich protein 2/3 gene deletions in African countries between January 2010 and December 2021 vary in methodological approaches such that application of results may not be generalized.

## RECOMMENDATION

Decisions and policies which depend on Pfhrp2/3gd reports, need to take into consideration the variation in research methods from one African country to another and results from one study may not necessarily be generalized to other Countries within the African continent

## REFERENCES

1. Bryan D, Silva N, Rigsby P, Dougall T, Corran P, Bowyer PW, et al. The establishment of a WHO Reference Reagent for anti-malaria (*Plasmodium falciparum*) human serum. *Malar J.* 2017;16(1):1–10.
2. Mbeye NM, ter Kuile FO, Davies MA, Phiri KS, Egger M, Wandeler G, et al. Cotrimoxazole prophylactic treatment prevents malaria in children in sub-Saharan Africa: systematic review and meta-analysis. *Trop Med Int Health.* 2014;19(9):1057–67.
3. Nambozi M, Malunga P, Mulenga M, Van Geertruyden JP, D'Alessandro U. Defining the malaria burden in Nchelenge District, northern Zambia using the World Health Organization malaria indicators survey. *Malar J.* 2014;13(1):1–7.
4. Phillips RS. Current status of malaria and potential for control. *Clin Microbiol Rev.* 2001;14(1):208–26.
5. Akinyi S, Hayden T, Gamboa D, Torres K, Bendezu J, Abdallah JF, et al. Multiple genetic origins of histidine-rich protein 2 gene deletion in *Plasmodium falciparum* parasites from Peru. *Sci Rep.* 2013;3.
6. Nega D, Dana D, Tefera T, Eshetu T. Prevalence and predictors of asymptomatic malaria parasitemia among pregnant women in the rural surroundings of Arbaminch Town, South Ethiopia. *PLoS One.* 2015;10(4):1–11.
7. Vanga-Bosson HA, Coffie PA, Kanhon S, Sloan C, Kouakou F, Eholie SP, et al. Coverage of intermittent prevention treatment with sulphadoxine- pyrimethamine among pregnant women and congenital malaria in Cte d'Ivoire. *Malar J.* 2011;10:1–10.
8. WHO. World Malaria Report 2020. World Health Organization. World Health Organization. 2020. 247 p.
9. McCaffery JN, Nace D, Herman C, Singh B, Sompwe EM, Nkoli PM, et al. *Plasmodium falciparum* pfhrp2 and pfhrp3 gene deletions among patients in the DRC enrolled from 2017 to 2018. *Sci Rep.* 2021 Dec 1;11(1).
10. Parr JB, Anderson O, Juliano JJ, Meshnick SR. Streamlined, PCR-based testing for pfhrp2- and pfhrp3-negative *Plasmodium falciparum*. *Malar J.* 2018 Apr 2;17(1).
11. Parr JB, Verity R, Doctor SM, Janko M, Carey-Ewend K, Turman BJ, et al. Pfhrp2 -Deleted *Plasmodium falciparum* Parasites in the Democratic Republic of the Congo: A National Cross-sectional Survey. *J Infect Dis.* 2017 Jul 1;216(1):36–44.
12. Bosco AB, Nankabirwa JI, Yeka A, Nsoby S, Gresty K, Anderson K, et al. Limitations of rapid diagnostic tests in malaria surveys in areas with varied transmission intensity in Uganda 2017-2019: Implications for selection and use of HRP2 RDTs. *PLoS One.* 2020 Dec 1;15(12 December 2020).
13. Pasquier G, Azoury V, Sasso M, Laroche L, Varlet-Marie E, Houzé S, et al. Rapid diagnostic tests failing to detect infections by *Plasmodium falciparum* encoding pfhrp2 and pfhrp3 genes in a non-endemic setting. *Malar J.* 2020 May 11;19(1).
14. Feleke SM, Reichert EN, Mohammed H, Brhane BG, Mekete K, Mamo H, et al. *Plasmodium*

- falciparum* is evolving to escape malaria rapid diagnostic tests in Ethiopia. Nat Microbiol. 2021 Oct 1;6(10):1289–99.
15. Laban NM, Kobayashi T, Hamapumbu H, Sullivan D, Mharakurwa S, Thuma PE, et al. Comparison of a PfHRP2-based rapid diagnostic test and PCR for malaria in a low prevalence setting in rural southern Zambia: Implications for elimination. Malar J. 2015;14(1).
  16. Plucinski MM, Herman C, Jones S, Dimbu R, Fortes F, Ljolje D, et al. Screening for Pfhrrp2/3-Deleted *Plasmodium falciparum*, Non-falciparum, and Low-Density Malaria Infections by a Multiplex Antigen Assay. J Infect Dis. 2019 Jan 9;219(3):437–47.
  17. Bakari C, Jones S, Subramaniam G, Mandara CI, Chiduo MG, Rumisha S, et al. Community-based surveys for *Plasmodium falciparum* pfhrrp2 and pfhrrp3 gene deletions in selected regions of mainland Tanzania. Malar J. 2020 Dec 1;19(1).
  18. Alemayehu GS, Blackburn K, Lopez K, Cambel Dieng C, Lo E, Janies D, et al. Detection of high prevalence of *Plasmodium falciparum* histidine-rich protein 2/3 gene deletions in Assosa zone, Ethiopia: implication for malaria diagnosis. Malar J. 2021 Dec 1;20(1).
  19. Nsoby SL, Walakira A, Namirembe E, Kiggundu M, Nankabirwa JI, Ruhamyankaka E, et al. Deletions of pfhrrp2 and pfhrrp3 genes were uncommon in rapid diagnostic test-negative *Plasmodium falciparum* isolates from Uganda. Malar J. 2021 Dec 1;20(1).
  20. Koita OA, Doumbo OK, Ouattara A, Tall LK, Konaré A, Diakité M, et al. False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene. Am J Trop Med Hyg. 2012;86(2):194–8.
  21. Nderu D, Kimani F, Thiong'o K, Akinyi M, Karanja E, Meyer CG, et al. PfHRP2-PfHRP3 diversity among Kenyan isolates and comparative evaluation of PfHRP2/pLDH malaria RDT with microscopy and nested PCR methodologies. Parasitol Int. 2018 Dec 1;67(6):793–9.
  22. Beshir KB, Sepúlveda N, Bharmal J, Robinson A, Mwanguzi J, Busula AO, et al. *Plasmodium falciparum* parasites with histidine-rich protein 2 (pfhrrp2) and pfhrrp3 gene deletions in two endemic regions of Kenya. Sci Rep. 2017;7(1):1–10.
  23. Menegon M, L'Episcopia M, Nurahmed AM, Talha AA, Nour BYM, Severini C. Identification of *Plasmodium falciparum* isolates lacking histidine-rich protein 2 and 3 in Eritrea. Infect Genet Evol. 2017 Nov 1;55:131–4.
  24. Berhane A, Anderson K, Mihreteab S, Gresty K, Rogier E, Mohamed S, et al. Major threat to malaria control programs by *Plasmodium falciparum* lacking histidine-rich protein 2, Eritrea. Emerg Infect Dis. 2018 Mar 1;24(3):462–70.
  25. Kozycki CT, Umulisa N, Rulisa S, Mwikarago EI, Musabyimana JP, Habimana JP, et al. False-negative malaria rapid diagnostic tests in Rwanda: impact of *Plasmodium falciparum* isolates lacking hrp2 and declining malaria transmission. Malar J. 2017 Mar 20;16(1).
  26. Gupta H, Matambisso G, Galatas B, Cisteró P, Nhamussua L, Simone W, et al. Molecular surveillance of pfhrrp2 and pfhrrp3 deletions in *Plasmodium falciparum* isolates from Mozambique. Malar J. 2017 Oct 16;16(1).
  27. Wurtz N, Fall B, Bui K, Pascual A, Fall M, Camara C, et al. Pfhrrp2 and pfhrrp3 polymorphisms in *Plasmodium falciparum* isolates from Dakar, Senegal: Impact on rapid malaria diagnostic tests. Malar J. 2013;12(1).
  28. Amoah LE, Abankwa J, Oppong A. *Plasmodium falciparum* histidine rich protein-2 diversity and the implications for PfHRP 2: Based malaria rapid diagnostic tests in Ghana. Malar J. 2016 Feb 18;15(1).
  29. Funwei R, Nderu D, Nguetse CN, Thomas BN, Falade CO, Velavan TP, et al. Molecular surveillance of pfhrrp2 and pfhrrp3 genes deletion in *Plasmodium falciparum* isolates and the implications for rapid diagnostic tests in Nigeria. Acta Trop. 2019 Aug 1;196:121–5.
  30. Kobayashi T, Sikalima J, Parr JB, Chaponda M, Stevenson JC, Thuma PE, et al. The search for *Plasmodium falciparum* histidine-rich protein 2/3 deletions in Zambia and implications for *Plasmodium falciparum* histidine-rich protein 2-based rapid diagnostic tests. Am J Trop Med Hyg. 2019;100(4):842–5.

31. Girma S, Cheaveau J, Mohon AN, Marasinghe D, Legese R, Balasingam N, et al. Prevalence and epidemiological characteristics of asymptomatic malaria based on ultrasensitive diagnostics: A cross-sectional study. *Clin Infect Dis*. 2019;69(6):1003–10.
32. Golassa L, Messele A, Amambua-Ngwa A, Swedberg G. High prevalence and extended deletions in *Plasmodium falciparum* hrp2/3 genomic loci in Ethiopia. *PLoS One*. 2020 Nov 1;15(11).
33. WHO. Protocol for estimating the prevalence of pfhrp2/pfhrp 3gene deletions among symptomatic falciparum patients with false negative RDT results. Geneva: World Health Organization; 2018

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