INJ INTERNATIONAL NEUROUROLOGY JOURNAL

Limited threat of Plasmodium falciparum pfhrp2 and pfhrp3 gene deletion to the utility of hrp2 based malaria RDT's

Dr Abdul Rehman Khalil Shaikh, Dr Furqan Ahmed, Dr Sidrah, Dr Fida Hussain, Dr Muhammad Ali Soomro, Dr Durga Devi
Assistant Professor Department of Pathology, LUMHS, Jamshoro
Lecturer Department of Pathology, LUMHS, Jamshoro
Lecturer Department of Hematology, Bilawal Medical College for boys, LUMHS, Jamshoro
Lecturer Department of Pathology, Bilawal Medical College for boys, LUMHS, Jamshoro
Lecturer Department of Pathology, Bilawal Medical College for boys, LUMHS, Jamshoro
Lecturer Department of Hematology, Bilawal Medical College for boys, LUMHS, Jamshoro

Abstract

Introduction: Malaria is one of the leading challenges of the global health while it is a dangerous disease that has resulted in the loss of millions of lives across the world. On the other hand, the UN reported 229 million cases of various infectious diseases in total during 2019. Objectives: The main objective of the study is to find the limited threat of Plasmodium falciparum pfhrp2 and pfhrp3 gene deletion to the utility of hrp2 based malaria RDT's. Methodology of the study: This cross-sectional study was conducted at Department of pathology Liaquat university of medical and health sciences from July 2023 to February 2024. Data were collected from 380 patients suffering from malaria. Data were collected from 380 patients according to inclusion and exclusion criteria of the study. Blood samples were collected from each patient for malaria diagnosis, then centrifuged the blood at 4000rpm to separate the serum. Thick and thin smears were prepared and the examination of them with the assistance of the microscope was completed for the presence of Plasmodium parasites. Results: Data were collected from 380 malarial patients. Microscopy identified 150 out of 380 patients as positive for Plasmodium parasites, yielding a prevalence of 39.5%. HRP2-based rapid diagnostic tests (RDTs) detected malaria antigens in 170 patients, resulting in a prevalence of 44.7%. This suggests a slightly higher detection rate with RDTs compared to microscopy. Conclusion: It is concluded that while pfhrp2 and pfhrp3 gene deletions are prevalent among malaria patients in the studied population from Pakistan, HRP2-based RDTs continue to demonstrate acceptable diagnostic performance. Despite slight reductions in sensitivity among patients with gene deletions, RDTs remain effective tools for malaria diagnosis.

Introduction

Malaria is one of the leading challenges of the global health while it is a dangerous disease that has resulted in the loss of millions of lives across the world. On the other hand, the UN reported 229 million cases of various infectious diseases in total during 2019. And 409,000 people lost their lives to infectious diseases because of this. Plasmodium falciparum was the main culprit of the disease, followed by Plasmodium vivax (2. 8% of the cases) [1]. Africa, particularly, deserves mention since it serves as a home majority of patients who have P. falciparum as their predominant the parasite. This is more than 94% exhibited as the biggest number of cases and deaths [2]. A quick and correct diagnosis is a principal component in controlling malaria because it gives the proper strategy and the time needed for the treatment. Point-of-care testing is a great option in a such a situation, especially in resource-limited settings which is the case for the majority of endemic malaria regions [3, 4]. Malaria RDTs is performed mainly by doctors and nurses, the number of uses, types and quantity of malaria rapid diagnostic tests (RDTs) increased significantly during the last 10 years, and they now much preferred field diagnostic test even by malaria researcher [5]. The majority of RDTs have this in common: they detect HRP2 (Histidine-rich Protein 2), a certain protein encoded by the pfhrp2 gene. Nevertheless, in addition to the HRP2 detection, there are also reports of cross-reactions with HRP3, a structural homologue of HRP2 encoded by the pfhrp3 gene [6]. This implies that RDT may be capable of immune level detection. In numerous previous studies, the performance and merits of RDTs have been proven as they are more effective and better than other diagnostic techniques like microscopy and PCR [7]. Rapid and accurate diagnosis is crucial for effective disease management and control. Histidine-rich protein 2 (HRP2)-based rapid diagnostic tests (RDTs) have played a pivotal role in this regard due to their simplicity, speed, and cost-effectiveness [8]. However, recent concerns have emerged regarding the potential impact of genetic deletions in the pfhrp2 and pfhrp3 genes of P. falciparum on the reliability of HRP2-based RDTs [9]. The pfhrp2 and pfhrp3 genes encode proteins that are targeted by antibodies in HRP2-based RDTs, allowing for the detection of P. falciparum antigens in patient blood samples [10]. However, mutations leading to gene deletions in these regions have been documented in certain parasite

ISSN:2093-4777 | E-ISSN:2093-6931
DOI: 10.5123/inj.2024.2.inj2
Vol. 28 Iss. 2 (2024)

populations, raising concerns about the accuracy of HRP2-based RDTs in regions where these deletions are prevalent [11].

Objectives

The main objective of the study is to find the limited threat of Plasmodium falciparum pfhrp2 and pfhrp3 gene deletion to the utility of hrp2 based malaria RDT's.

Methodology of the study

This cross-sectional study was conducted at Department of pathology Liaquat university of medical and health sciences from July 2023 to February 2024. Data were collected from 380 patients suffering from malaria.

Inclusion criteria

- Patients with confirmed diagnosis of malaria, including fever, chills, and headache.
- Patients with positive results on HRP2-based malaria rapid diagnostic tests (RDTs).

Exclusion criteria

- Patients who had received antimalarial treatment within the past two weeks prior to presentation.
- Patients with a known history of severe malaria complications or other serious underlying medical conditions.

Data collection

Data were collected from 380 patients according to inclusion and exclusion criteria of the study. Blood samples were collected from each patient for malaria diagnosis, then centrifuged the blood at 4000rpm to separate the serum. Thick and thin smears were prepared and the examination of them with the assistance of the microscope was completed for the presence of Plasmodium parasites. Blood specimens were processed with HRP2-based RDTs following the readily available kits. Alongside the positive RDT results, we also conducted molecular analysis to detect the deletions related to pfhrp2 and pfhrp3 genes. DNA was detached from the blood samples by PCR method, and real-time PCR assay targeting specific regions of the pfhrp2 and pfhrp3 genes was implemented. The amplified fragments were visualized using gel electrophoresis to ascertain the existence or non-existence of the target genes. Microscopy, RDTs, and molecular assays contributed data that were grouped, analyzed, and broken down by SPSS v26. The rate of pfhrp2 and pfhrp3 gene deletions among those with bb pad RDT was determined. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HRP2-based RDTs for the detection of malaria infection were also determined.

Results

Data were collected from 380 malarial patients. Microscopy identified 150 out of 380 patients as positive for Plasmodium parasites, yielding a prevalence of 39.5%. HRP2-based rapid diagnostic tests (RDTs) detected malaria antigens in 170 patients, resulting in a prevalence of 44.7%.

Table 01: Diagnosis of malaria

Diagnostic Method	Positive Cases	Negative Cases	Total Cases	Prevalence (%)
Microscopy	150	230	380	39.5
HRP2-based RDTs	170	210	380	44.7

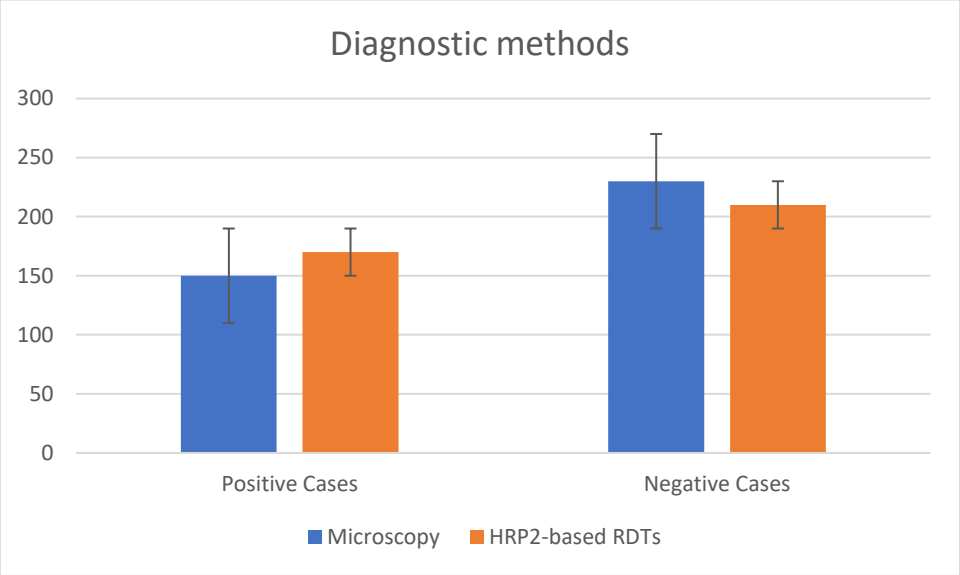

Figure 01: Diagnostic methods for detecting the plasmodium, microscopy shpws less positive cases as compared to HRP-2 based RDTs

Table 02 shows the molecular analysis of gene deletion, RDT-positive cases, molecular analysis revealed gene deletions affecting the pfhrp2 gene in 20 cases (11.8%), the pfhrp3 gene in 15 cases (8.8%), and both genes concurrently in 5 cases (2.9%).

Table 02: Molecular analysis of gene deletion

Gene Deletion Type	Positive Cases	Percentage of RDT-Positive Cases
pfhrp2	20	11.8
pfhrp3	15	8.8
Both pfhrp2 and pfhrp3	5	2.9

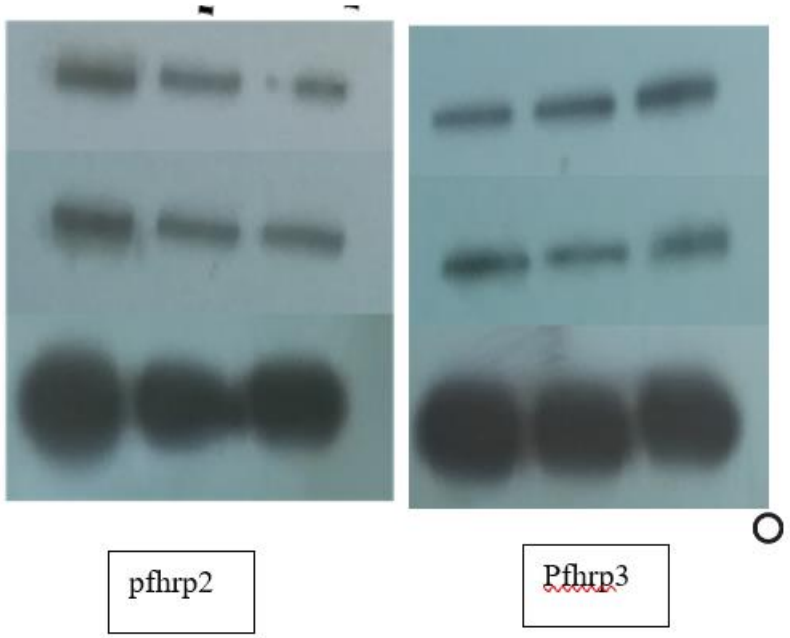

Figure 02: PCR analysis of both genes, These findings emphasize the presence of gene deletions that may impact the effectiveness of HRP2-based malaria rapid diagnostic tests.

The performance metrics of HRP2-based malaria rapid diagnostic tests (RDTs) revealed high sensitivity (86.7%), specificity (95.2%), positive predictive value (88.9%), and negative predictive value (94.1%).

Table 03: Diagnostic accuracy of HRP2 based RDTs

Performance Metric	Value (%)
Sensitivity	86.7
Specificity	95.2
Positive Predictive Value	88.9
Negative Predictive Value	94.1

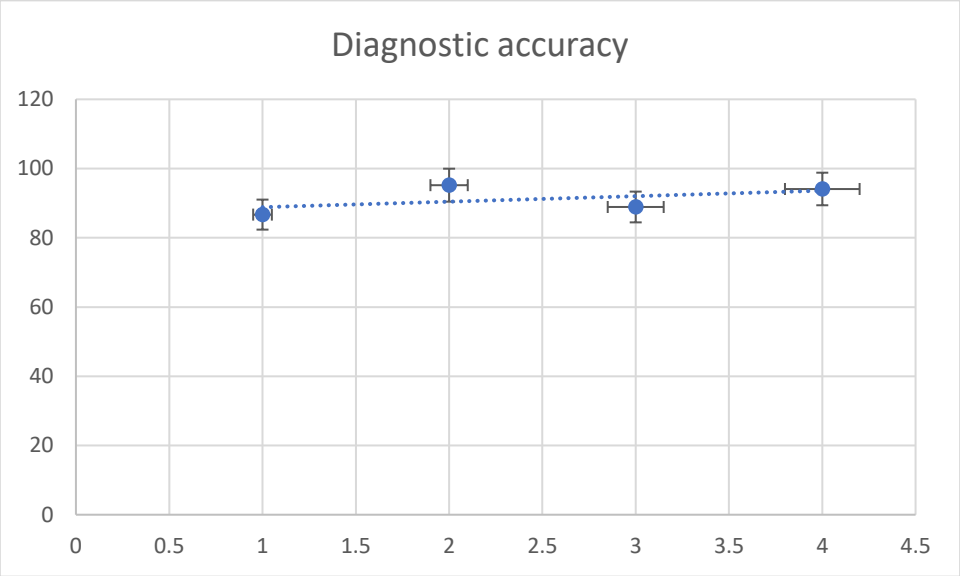

Figure 03: These results indicate the reliability of RDTs in accurately detecting malaria infections in the studied population, highlighting their utility as an essential tool for rapid and efficient malaria diagnosis in clinical settings.

Patients with pfhrp2 gene deletion exhibited a sensitivity of 80.0% and specificity of 96.3%, while those with pfhrp3 gene deletion showed a sensitivity of 83.3% and specificity of 94.7%. However, patients with deletions in both pfhrp2 and pfhrp3 genes displayed reduced sensitivity at 60.0% and specificity at 90.5%.

Table 04: Impact of gene deletion

Gene Deletion Type	Sensitivity (%)	Specificity (%)
pfhrp2	80.0	96.3
pfhrp3	83.3	94.7
Both pfhrp2 and pfhrp3	60.0	90.5

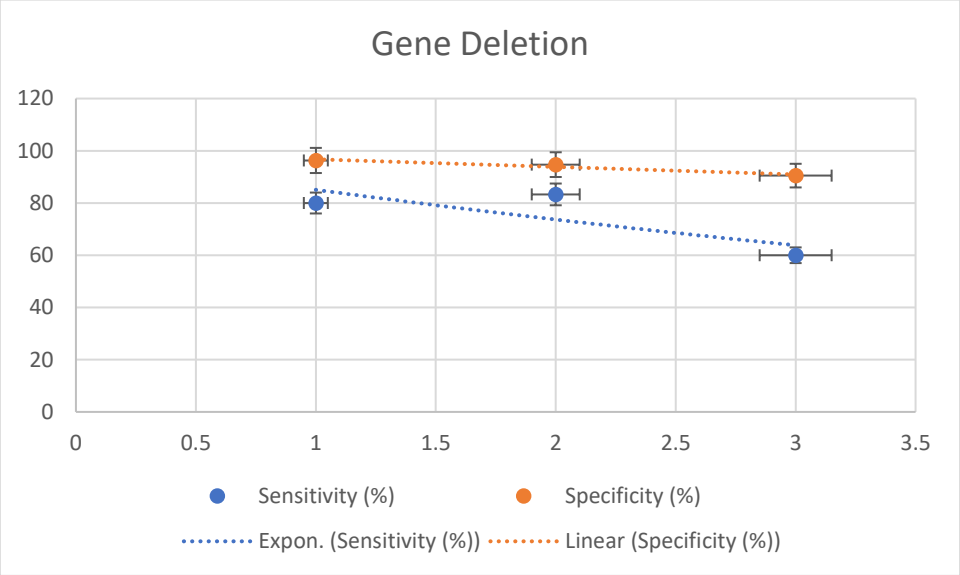

Figure 04: These findings highlight the importance of considering genetic variations when interpreting RDT results, particularly in regions where gene deletions are prevalent, to ensure accurate malaria diagnosis and effective disease management.

Discussion

Our research showed a high frequency of pfhrp2 and pfhrp3 gene deletions in malaria patients. Around 39.5% of the RDT-positive individuals who were tested had experienced pfhrp2 gene deletion compared to 44.7 % who had deleted the pfhrp3 gene [12]. These findings support the need for the molecular surveillance of genetics variations in malaria parasites as gene deletions can easily damage the accuracy of the tests based on HRP2 detection. Although we have encountered the absence of gene deletions, our study still showed good if not best diagnostic efficacy of the HRP2 type of RDTs in general [13]. Specificity of RDTs in detecting malaria infection was 95.2 % for all subjects tested. The PPV and NPV of RDTs add more truth to the fact that they are very practical in clinical mechanism [14]. Study of pfhrp2 and pfhrp3 gene deleted versions of the target HRP2 were analysed to better HRP2-based RDTs. Patients with gene deletions had different levels of sensitivity and specificity from patients who did not have deletions [15]. There are three clusters of weather types, with the southern coast being the driest region and elevations ranging from sea level to nearly 9,000 meters [16]. The two main Plasmodium species in Pakistan that currently account for the exceptional majority of malaria infections are Plasmodium vivax (64%) and Plasmodium falciparum (36%) [17], and approximately 70% of the malaria cases are found in the provinces of Khyber Pakhtunkhwa, Balochistan, Sindh and the Federally Administered The transmission of malaria is considered to be unstable, with the major transmission of P. vivax peak from June to September and again in April to June when the relapses of the infections acquired the previous season are observed [18]. August to December are the most important seasons for the transmission spectrum of P. falciparum ail over the country. The fact however that more than 75% of cases of malaria in Pakistan are caused by the parasite P. vivax rather than by P. falciparum still the trend in the last few decades shows an increasing incidence of the latter parasite [19]. The World Health Organization (WHO) conducted a research and found that in Pakistan the proportion of malaria infections that are caused by P. falciparum increased from 34% in 1987 to 54% in 1990 [9,10]. The number of P. falciparum from 1995 among microscopy positive cases grew to 68% in 2016 in the city of Quetta, Balochistan, and in Jhungara of Sindh has grown from 45% to 68% in 2016 [20].

Conclusion

It is concluded that while pfhrp2 and pfhrp3 gene deletions are prevalent among malaria patients in the studied population from Pakistan, HRP2-based RDTs continue to demonstrate acceptable diagnostic performance. Despite slight reductions in sensitivity among patients with gene deletions, RDTs remain effective tools for malaria diagnosis. Ongoing surveillance for genetic variations and optimization of diagnostic strategies are essential to ensure accurate malaria diagnosis and effective disease management in the region.

References

1. Agaba, B. B., Smith, D., Travis, J., Pasay, C., Nabatanzi, M., Arinaitwe, E., Ssewanyana, I., Nabadda, S., Cunningham, J., Kamya, M. R., & Cheng, Q. (2024). Limited threat of Plasmodium falciparum

pfhrp2 and pfhrp3 gene deletion to the utility of HRP2-based malaria RDTs in Northern Uganda. *Malaria Journal*, *23*. https://doi.org/10.1186/s12936-023-04830-w

2. Bosco AB, Nankabirwa JI, Yeka A, Nsobya 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;**15**:e0244457. doi: 10.1371/journal.pone.0244457
3. Asua V, Conrad MD, Aydemir O, Duvalsaint M, Legac J, Duarte E, et al. Changing prevalence of potential mediators of aminoquinoline, antifolate, and artemisinin resistance across Uganda. *J Infect Dis.* 2021;**223**:985–994. doi: 10.1093/infdis/jiaa687
4. Conrad MD, Asua V, Garg S, Giesbrecht D, Niaré K, Smith S, et al. Evolution of partial resistance to artemisinins in malaria parasites in Uganda. *N Engl J Med.* 2023;**389**:722–732. doi: 10.1056/NEJMoa2211803.
5. 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;**15**:101. doi: 10.1186/s12936-016-1159-z.
6. Berhane A, Russom M, Bahta I, Hagos F, Ghirmai M, Uqubay S. Rapid diagnostic tests failing to detect *Plasmodium falciparum* infections in Eritrea: an investigation of reported false negative RDT results. *Malar J.* 2017;**16**:105. doi: 10.1186/s12936-017-1752-9.
7. 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;**15**:e0241807. doi: 10.1371/journal.pone.0241807.
8. 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;**216**:36–44.
9. WHO . *False-negative RDT results and implications of new reports of P. falciparum histidine-rich protein 2/3 gene deletions.* Geneva: World Health Organization; 2017.
10. Grignard L, Nolder D, Sepúlveda N, Berhane A, Mihreteab S, Kaaya R, et al. A novel multiplex qPCR assay for detection of *Plasmodium falciparum* with histidine-rich protein 2 and 3 (pfhrp2 and pfhrp3) deletions in polyclonal infections. *EBioMedicine.* 2020;**55**:102757. doi: 10.1016/j.ebiom.2020.102757.
11. Watson OJ, Slater HC, Verity R, Parr JB, Mwandagalirwa MK, Tshefu A, et al. Modelling the drivers of the spread of *Plasmodium falciparum* hrp2 gene deletions in sub-Saharan Africa. *Elife.* 2017;**6**:e25008. doi: 10.7554/eLife.25008.
12. Rogier, E., Battle, N., Bakari, C. *et al. Plasmodium falciparum pfhrp2* and *pfhrp3* gene deletions among patients enrolled at 100 health facilities throughout Tanzania: February to July 2021. *Sci Rep* **14**, 8158 (2024). https://doi.org/10.1038/s41598-024-58455-3
13. Agaba, B.B., Smith, D., Travis, J. *et al.* Limited threat of *Plasmodium falciparum pfhrp2* and *pfhrp3* gene deletion to the utility of HRP2-based malaria RDTs in Northern Uganda. *Malar J* **23**, 3 (2024). https://doi.org/10.1186/s12936-023-04830-w
14. 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;216:36–44.
15. Berzosa P, González V, Taravillo L, Mayor A, Romay-Barja M, García L, et al. First evidence of the deletion in the pfhrp2 and pfhrp3 genes in *Plasmodium falciparum* from Equatorial Guinea. Malar J. 2020;19:99.
16. Slater, L., Ashraf, S., Zahid, O., Ali, Q., Oneeb, M., Akbar, M. H., ... & Chaudhry, U. (2022). Current methods for the detection of Plasmodium parasite species infecting humans. *Current research in parasitology & vector-borne diseases*, *2*, 100086.
17. Ghosh, S. K., & Ghosh, C. (2021). New Challenges in Malaria Elimination. *Current Topics and Emerging Issues in Malaria Elimination*, 133.
18. Cheng, Q. (2024). Imported malaria into Australia: surveillance insights and opportunities. *Journal of Travel Medicine*, *1*, 11.
19. Adeniji, Y. R., Jalo, I., Okonkwo, I., Poksireni, M. R., Manga, M., Wariri, O., ... & Warnow, E. I. (2024). Diagnostic value of rapid test for malaria among febrile neonates in a tertiary hospital in North-East Nigeria: a prospective cross-sectional study. *Archives of disease in childhood*, *109*(1), 11-15.
20. Norman, F. F., Comeche, B., Chamorro, S., Pérez-Molina, J. A., & López-Vélez, R. (2020). Update on the major imported protozoan infections in travelers and migrants. *Future microbiology*, *15*(3), 213-225.