Review

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.1436244
J Health Sci Med. 2024;7(2):236-243

Meta-analytic review on the impact of factors that affect performance of malaria rapid diagnostic test in Africa

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Cite this article as: Ongonda JK, Ayieko C, Miheso S. Meta-analytic review on the impact of factors that affect performance of malaria rapid diagnostic test in Africa. *J Health Sci Med.* 2024;7(2):236-243.

Received: 13.02.2024 ◆ **Accepted**: 13.03.2024 ◆ **Published**: 25.03.2024

ABSTRACT

Timely, accurate diagnosis and treatment has improved malaria case management. Malaria Rapid Diagnostic Test (mRDT) kits are largely used in malaria diagnosis. Their performance is compromised by factors related to gene deletions, parasite density, quality of the kit, poor storage conditions and end-user inefficiencies hence diagnosis gives either positive, negative, false negative (FN) or false positive (FP) which defines consequent management strategies. This review assessed reports on prevalence of the Plasmodium falciparum histidine rich protein 2/3 (Pfhrp2/3) gene deletions in malaria infected populations in Africa and the risk of mRDT failure to identify malaria positive cases. Preferred Reporting Items for Systematic Meta-Analysis (PRISMA) statement was used for data collection. Literature search was done using Google and Mendel search for data published in a malaria journal, Journal of infectious diseases, scientific reports, Annals of Ibadan postgraduate medicine, and BMC journals published between 2019 and 2023. Fifty eight reports were identified were screened and tested for eligibility. Majority of studies described the consistent use of Pfhrp2/3 mRDT for malaria diagnosis in rural health facilities in Africa and nine reports met inclusion criteria for review. Five of them certified the world health organization's sample criteria of 'more than 350 sample' to estimate the prevalence of Pfhrp2/3 gene deletions leading to declaration of false negative results of which one study posted FN outcome resulting from these deletions. Four out of nine studies did not meet this WHO criterion. This review affirmed presence of Pfhrp2/3 gene deletions challenges in Africa though other countries recorded the converse. Data was pooled using random effect models with Odds ratio and 95% confidence limit. The prevalence of the gene deletions was heterogeneous, ranging from 0% to 78.1%. The review found that an average prevalence of Pfhrp2/3 deletion as 26.2%. This was above the WHO standard recommended declaration value of 5%.; a factor that demonstrated setback to the use of mRDT in malaria endemic regions. Therefore alternative methods should be used where aspersions are cast on outcome of mRDT for it will help improve malaria treatment, tracking and management.

Keywords: Malaria diagnosis, Pfhrp2/3 gene deletions, malaria RDT

INTRODUCTION

Malaria is still a global health challenge killing thousands of people albeit governments efforts in investing heavily into its diagnosis, treatment and control.[1] This situation is worse in sub-Saharan Africa where specifically, children below five (5) years of age bear the greatest burden of morbidity and fatalities resulting from malaria infection.[2-4] To control and contain the malaria, the World Health organization has recommended the strategy of three TTTs (Testing, Treating and Tracking) of the malaria.[5-9] The TTT controls haphazard anti-malarial consumption which by extension reduces emergence of resistance to antimalarial drugs, declining malaria transmission in once considered malaria endemic areas and ultimately reduces pressure on available malaria Rapid Diagnostic Test (mRDTs) in resource constrained facilities.[10]

Initially, malaria diagnosis was done by Microscopes but lack of the required infrastructure and expertise in certain facilities has led to preferential use of malaria Mrdt.[11-13] Currently, mRDTs accounts for over 75% of malaria diagnosis in rural health facilities in Africa.[2] Facilities constrained by diagnostic infrastructure use physical examination, despite the risk of misdiagnosis, to manage malaria. This maintains a cycle of illness if not pointed out by alternative diagnosic methods and later be subjected to treatment.[1,8,10] Therefore, mRDTs offer a great potential for quick and convenient malaria diagnosis, especially in rural settings lacking alternative diagnostic methods.[7,14,15]

The mRDT detect malaria parasite antigens that are circulating in the blood stream.[10,16] The parasites' antigen

markers define the specific RDTs and hence Lactate dehydrogenase (LDH)-based and aldolase-based RDTs are said to be pan specific because they detect all human malaria species.[17] Plasmodium falciparum antigens are the most prevalent hence mRDTs specific for Pfhrp2/3 antigens are the most used in malaria diagnosis. However, the use mRDTs specific for Pfhrp2/3 antigens is sometimes challenged by occurrence of false positive or false negative results which can result to malaria misdiagnosis hence inappropriate malaria drug use or treatment failure respectively.[18]

There are many factors that influence the accurate performance of mRDT. They include Pfhrp2/3 gene deletions, mRDT sensitivity, quality of the mRDT cassettes, low parasite densities, susceptibility to the prozone effect, cross-reaction between plasmodium antigens and detection monoclonal antibodies, susceptibility to heat and humidity.[19] World health organization has called for a review and change in policy direction on malaria testing, treating and tracking in Africa.[7,8,20] Despite reports confirming that malaria transmission is declining in Africa, spatial and temporal mutability of malaria poses new challenges that impact negatively on malaria control programmes.[8,21]

Plasmodium falciparum hrp2/3 RDTs have been broadly used for diagnosis of malaria, especially in facilities lacking well established infrastructure for diagnosis. The emergene of Plasmodium falciparum mutant types has, ironically, led to mRDT negative results for malaria positive cases![4,22] This is a challenge to malaria treatment and control. The Pfhrp2/3 gene deletions protects the malaria parasite antigens causing them to evade detection by the monoclonal antibodies (Mab) of the Immunochromatographic test (ICT) resulting to false negative (FN) cases.[4,7,21,23,24] False negative patients may miss out on malaria treatment (unless confirmed otherwise). Heterogeneity in Pfhrp2/3 gene deletion prevalence could be pronounced even in different regions within the same Country. In Eritrea, North West area has a high prevalence of these deletions than the South and South west; just like the Anseba zone which also had the higher prevalence of Pfhrp2/3 deletions than the Gash Barka zones. In such like a set-up, it makes reliability of Pfhrp2/3 mRDTs not very safe.[14,25] Pfhrp2/3 antigens may also be detected in the blood circulation long after malaria parasites have been cleared off after treatment.25 The antigens will be detected by the mRDT, as if the parasites were still present. This leads to false positive (FP) malaria test interpretation and if not confirmed otherwise, may lead to consumption of anti-malarial drugs by healthy subjects.[16]

Studies have shown that, there are more Pfhrp2/3 false negative results detected at the beginning of a rainy season than during the rainy season. The above trend is reversed when the rains subside.[14,26] This is an indication that more of the expression of Pfhrp2/3 deletions are linked to low rainfall and consequently leads to low intensity of infection (Super infection).[4] Since Malaria seasonality and intensity varies widely within and between epidemiological set-ups, the choice and timing for intervention methods for malaria control should vary.[4,22] Low rainfall increases the chances of getting Pfhrp2/3 gene deleted parasites and consequently increased chance of getting false negative mRDT outcome.

The quality of the mRDT cassettes does affect their use as well since it may give inaccurate test outcome. The World Health Organisation has advised on storing RDTs kits centrally in an air-conditioned area at a temperature less than 30°C to maintain its quality, an assurance to its accuracy.[4,14] Care should also be taken to minimise the kits degradation, especially during transportation.[27] Temperatures that exceed the recommended levels are bound to compromise the quality of the test results as well.[4] The choice of RDTs should depend on the expected field conditions and end-users should be ready to control conditions in the test kit supply chain especially in the tropical and sub-tropical climates.[14,28-32] RDT Kit labels and any instructional material packed should be in tandem with the internationally accepted standard operating procedure otherwise it can compromise the mRDT accurate outcome thus slowing down malaria control in Africa.[33]

Malaria parasite transmission intensity affects the performance of mRDT. During malaria surveillance by mRDT, P. falciparum antigens are detected and isolated for malaria treatment.[34] However, there are false negative (FN) cases that are found during the use of mRDT especially when the parasites are in very low densities (<100 asexual parasites/µl or <0.002% of red blood cells infected). Some FN cases can also be detected at a very high parastaemia (hyperparastaemia) caused by the prozone effect. Hyperparastaemia is defined by the WHO as infections of >5% of red blood cells.[33] The prozone effect (sometimes referred to as high doses-hook phenomenon) is caused by a higher concentration of antigens than antibodies or the converse.[16,33,35] In this respect, high concentration of Pfhrp2/3 antigens causes exhaustion of the antibodies hence they fail to reach their expected reaction point on the mRDT consequently there will be no signal hence interpreted as a negative malaria status.[33,35-37]

The inconsistencies in mRDT outcomes caused by various factors including Pfhrp2/3 deletions, malaria parasite transmission intensity, parasite densities, host gender dynamics and seasonality have challenged malaria management strategies and consequently have

shown a knowledge gap. Review of the factors that influence performance of mRDT in Africa has not been done and hence its datais lacking. This, therefore, calls for more studies on the above factors to help realign data generated for a wider understanding and provide better malaria management strategies. This study has, therefore done a meta-analytic review of the effect of Pfhrp2/3 gene deletions to the performance of mRDT in Africa.

METHODOLOGY

Selection criteria and Search Strategy

We used Preferred Reporting Items for Systematic Meta-Analysis (PRISMA) statement to collect data from the following screened journals/sources: Malaria Journals, Parasite and Vectors, Trends in Parasitology, Malaria Microbiology and International Journals of Infectious diseases. Other journals were from Clinical Microbiology, BMC Public Health, American Journal of tropical diseases and BMJ Global Health. The five search words used for the above data extraction were Malaria RDTs, parastaemia, Pfhrp2/3 deletions, malaria seasonality and false negatives. We searched using titles, abstracts and full papers to extract the information. Only three categories of the sources fitted our prescription and they are 3 Malaria journals, a BMC public health journal and a Journal of infectious diseases. This study was approved by National Commission for Science, Technology&Innovation (NACOSTI), Kenya and the Maseno University Scientific and Ethical Review committee.

All the studies reviewed were cross-sectional surveys which aimed at assessing the prevalence of the Pfhr2/3 gene deletions and their effect to FN malaria test outcome as shown in Figure 1. These Studies were selected based on originality of the work, availability of primary data, study done in Africa and reported in 2019 or later. These studies assessed the accuracy of the mRDTs in the peripheral blood as referenced to microscopy (gold standard) and the polymerase chain reaction 'the molecular aspect' of malaria diagnosis. Participants enrolled into these studies were of all ages, infected with malaria, had no co-infections and were residents in Africa.

Extraction of data and Its Analysis

Variables under review were; authors, Country/Area/site, study design, Sample population, age, malaria diagnostic method, Phrp2/3 deletions prevalence, statistical tools used, malaria status, the type of publication, the type of journal published in and the year of publication. Malaria prevalence heterogeneity was established in all the studies under review. Chi-Square tests with 95% confidence interval and odds ratio (OD) were used to assess the risk of malaria false negative (FN) resulting from effect of Pfhrp2/3 gene deletions, malaria seasonality, status of the

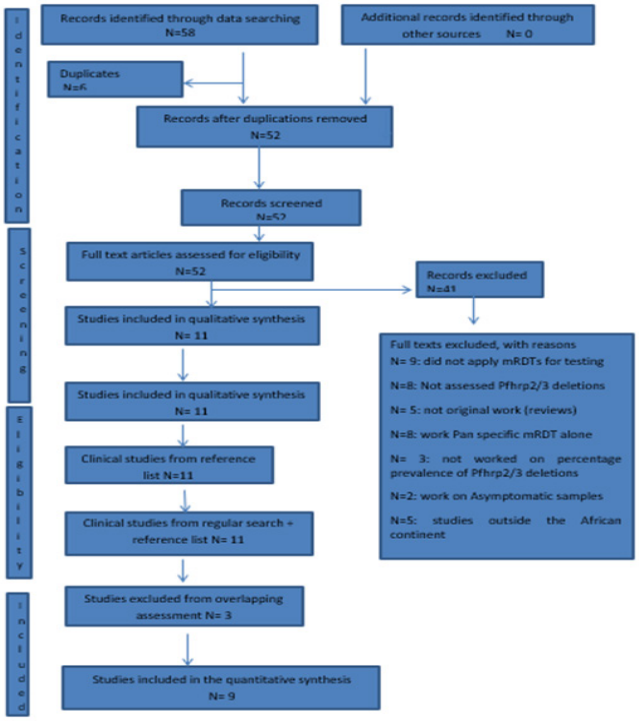

Figure 1. Analysis for inclusion of articles published for review

mRDT Kit and transmission intensity on performance of mRDT in nine (9) studies under review.

We identified Fifty eight (N=58) articles from research data with no additional article from any other source as shown in Table 1. There were six (6) duplicates from the research data which were removed from the study. Screening of the remaining fifty one (52) articles excluded forty four (41) articles from the review as they did not meet the inclusion threshold set by the team. Only eight (8) full articles were used for this review.[5,7,25,26,28,32,38,39] A total of 4482 subjects from these articles were assessed for FN status in this review study. Of the nine (9) tabulated, eight (8) studies captured the prevalence of FN cases in their study samples while one (1) article, despite assessing factors affecting mRDT performance, did not give the prevalence of FN in Nigeria.19 The Pfhrp2/3 false negative mRDT results studies in the eight countries of Africa revealed the following FN percentage prevalence: Ethiopia (78.10%), Eritria (57.90%), Tanzania (2.80%), Kenya (0.0%), Sudan (33.30%), Uganda (6.40%), DR Congo (0.27%) and Angola (0.40%) were recorded.

These studies did not lay emphasis on age as a parameter for exclusion or otherwise. Many reports on Pfhrp2/3 gene deletions in Africa were lacking and more so, those reported in the year 2019 or later were even fewer. Due to laboratory infrastructural deficiencies in malaria diagnosis (Microscopists/Microscops/PCR), the use of mRDT is so common in rural health facilities in Africa.[15,32] The thought of mRDT failure is worrying as it will wholesomely affect malaria treatment, control and

Table 1. Guiding parameters in the articles for the systemic review on impact of Pfhrp2/3 gene deletions

	Nderu et al., 2019	Michael, Olamadegun and Falade 2021	Nsobya et al., 2021	McCaffery et al., 2021	Golassa et al., 2020	Mihreteab et al. 2021	Musa et al., 2019	Thomson et al., 2019	Plucinskiet al., 2019
Design	Survey	Survey	Cross sectional survey	Cross sectional survey	Survey	Cross sectional survey	Survey	Survey	Cross sectional survey
Sample Population	400	226	1493	1109	64	715	59	176	466
Age	N/A	≤30 months	All Ages	All Ages	All Ages	All Ages	All Ages	All Ages	All Ages
Data collection site	Kenya	Nigeria	Uganda	DRC	Ethiopia	Eritrea	Sudan	Tanzania	Angola
Malaria Pfhrp2/3 confirmation method	RDT, Microscopy and PCR	RDT, Microscopy and PCR	Microscopy PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR	RDT Microscopy and PCR
Statistical tools	Chi Square test %ages	SD & Geometric mean and CI	Chi Square test %ages, 95% C	Chi Square test %ages, 95% CI	Chi Square test %ages, 95% C	Chi Square test %ages, 95% CI	Chi Square test %ages, 95% C	Chi Square test %ages, 95% CI	Chi Square test %ages, 95% CI
Deletions Prevalence	1.0%	N/A	6.40%	0.27%	78.10%	57.90%	33.30%	2.80%	0.40%
Recommendations	More work on the Pfrhp2 pfhrp3 deletions	mRDT should not be used for monitoring antimalarial therapies	More work on Pfhrp2/3 gene deletions	Continuos work on pfhrp2/3 deletions encouraged	Continued surveillance in Eritrea & environs	More research on the Pfhrp2/3 deletions	More research on Pfhrp2/3 deletions	More research on Pfhrp2/3 deletions	More research on Pfhrp2/3 deletions
Publishers	Scientific Reports	Ann of Ibadan Postgraduate Medicine	Malaria Journal	Scientific Reports	Plos One	Scientific Reports	BMC Journals	Jorunal of Infectious Diseas	Jorunal of Infectious Diseas

management. Therefore, more cross-sectional surveys on impact of factors that affect malaria rapid diagnostic test performanceare is paramount to help advice governments on possibility of re-looking at the malaria testing policies and procedures of managing either malaria positive patients or negative individuals.

In Africa, the mean of the sample population from data above for Pfhrp2/3 gene deletions was 560.25 while the mean percentage prevalence Pfhrp2/2 deletion was 22.5%. as shown above in Table 2. The mean percentage prevalence is far beyond the recommended baseline of 5% (22.5%≥ 5%).33 Hence, it is advisable that for negative mRDT results, confirmatory tests should ensue to flag out FN cases. WHO recommends change in policy on Pfhrp2/3 mRDT use if deletion prevalence value is ≥5% assessed from a cross-sectional survey with a sample population of 350 or more.34 Otherwise, there should be confirmatory tests to rule out FN in Pfhrp2/3 mRDT outcome.[4,7]

The 95% confidence interval analysis and explanations: The Confidence interval (CI) refers to the range of values which we can be 95% confident includes the mean of the population from which the sample was drawn. The values were calculated at 95% and presented as shown in Table 3.

Table 2. The mean values for samples population and percentage prevalence Pfhrp2/3 gene deletions

Country	Sample population	%age Pfhrp2/3 deletions	CI
Kenya	400	1	.0037424 .0264429
Uganda	1493	6.4	.052805 .0777828
Ethiopia	64	78.1	.6601466 .8678388
Eritria	715	57.9	.5423822 .6148096
Sudan	59	33.3	.2273939 .4718864
Tanzania	176	2.8	.0117717 .0669674
DR Congo	1109	0.27	.0117717 .0669674
Angola	466	0.4	.0010682 .0170782
Mean	4482/8=560.25	180.17/8=22.5	

Analysis revealed that 1% of the population in Kenya had Pfhrp 2/3 gene deletions when tested by mRDT kit with a 95% CI (0.37-2.64), 6.4% for Uganda, 95% CI (5.28-7.78), 78.1% for Ethiopia, 95% CI (66.01-86.78), 57.9% for Eritrea, 95% CI (54.24-61.48), 33.3% for Sudan, 95%

Table 3. Confidence interval values for the prevalence of malaria across selected countries in Africa

Country	No of Cases	CI	%CI
Kenya	4	.0037424 .0264429	0.37 2.64
Uganda	96	.052805 .0777828	5.28 7.78
Ethiopia	50	.6601466 .8678388	66.01 86.78
Eritrea	414	.5423822 .6148096	54.24 61.48
Sudan	20	.2273939 .4718864	22.74 47.19
Tanzania	5	.0117717 .0669674	1.18 6.7
DR Congo	3	.0117717 .0669674	1.18 6.7
Angola	2	.0010682 .0170782	0.11 1.71

CI (22.74-47.19), 2.8% for Tanzania, 95% CI (1.18-6.7), 0.27% for DRC Congo, 95% CI (1.18-6.7) and 0.4% for Angola, 95% CI (0.11-1.71). The statistics above shows the average percentage of Pfhrp 2/3 gene deletions in Kenya sample lies between 0.37% and 2.64%. Angola had the least value of between 0.11% and 1.71 % while Ethiopia had the highest value of between 66.01% and 86.78%.

Kenya had previously reported a prevalence of 10% however in 2017, a new study which was included in this analysis (from the year 2019 and above) reported one per cent (1%) for the Pfhrp2/3 gene deletions, a concept pointed to, may be, challenges in the methodologies used for the two studies.[15,40] Uganda and Sudan reported 6.4% and 33.3% Pfhrp2/3 gene deletions respectively.[5,26] while Ethiopia and Eritrea, neighbouring countries, recorded 78.1% and 57.9% gene deletions respectively as shown in Figure 2. The two presented the highest and second highest percentage of Pfhrp2/3 gene deletions in Africa.[25,28] There are reports that the prevalence of Pfhrp2/3 deletions could even be hhigher in the North African zones.6,25 Tanzania, Angola and DR Congo had 2.8%, 0.4% and 0.27% respectively; a trend whose percentage seems to be diminishing the Pfhrp2/3 deletions Southwards of the African continent.[15,39,40]

DISCUSSION

Analysis of Demographic Health Surveys (DHS) and Malaria Indicator Surveys (MIS) has shown that the prevalence of FN-RDT is spatially heterogeneous. It occurs frequently in low malaria transmission areas and also in urban areas, areas that were initially thought to be experiencing reduced rate of malaria infection. The analysis also exposes other factors, not related directly to the parasite, which does affect mRDT performance

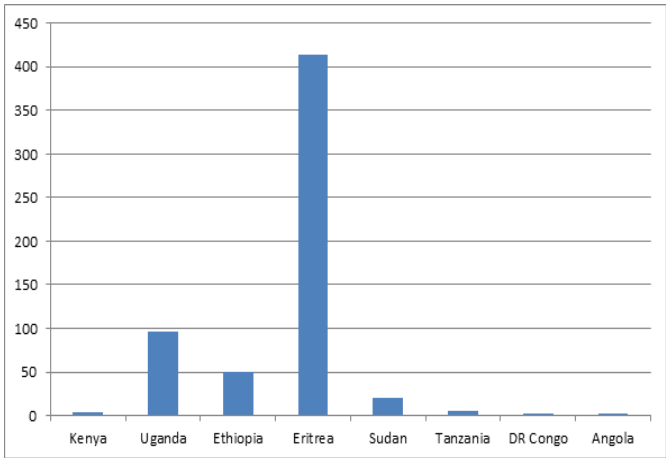

Figure 2. Prevalence of Pfhrp2/3 gene deletions

as well. False Negative-RDT results are not a preserve of mRDT product testing alone but involve other factors related to parasite density, quality of mRDT, environmental challenges up to even end-user capacity or lack of it thereof. In lieu of the above, mRDT procedure and analysis calls for accurate interpretation of the test outcome to enhance people's confidence towards their use and sustained good performance towards malaria community surveillance.

Countries that neighbour those with high percentage of Pfrp2/3 gene deletion are at risk of FN challenge-spill over. Kenya, which neighbours Ethiopia and Sudan with 78.1% and 33.3% Pfhrp2/3 gene deletions respectively may be exposed to FN challenges caused by these deletions since there is cross-border interaction's by the residents of either country.[5,28] The WHO has since then recommended routine surveillance of the Pfhrp2/3 gene deletions in such like countries to help inform national malaria diagnosis policies aimed at controlling the spread of malaria.[4,34]

In malaria endemic zones, there could be as much as FN-RDT (negative for RDT but positive for microscopy or any other molecular diagnosis) as there are FP-RDT (pfhrp2/3 antigens present well after parasite clearance after treatment) resulting from antigenaemia. The scenario above may misguide service providers to either scale down malaria treatment because of negative (FN) status or scale up treatment because of supposedly 'high malaria infections' (FP) while the converse is true. Either way, this review proposes alternative diagnostic methods to be used to confirm or disqualify results of FN or FP for proper intervention methods.[7] When analysing FN-RDT results and benchmarked with the gold standard (Microscopy) it is always assumed that the gold standard has no flaws, a concept that is challenged by presence of poorly processed blood smears, poor quality reagents, gaps in operator training and high workload for the operator to remain consistently accurate. This calls

for strict checks on standards of the whole process of microscopy to ascertain quality of test results. Molecular diagnosis (PCR) can also be used as a confirmatory test for the presence or absence of parasites.

There are three types of malaria RDT based on the antigens targeted. Both aldolase targeting and Lactate Dehydrogenase targeting mRDTs are referred to as pan specific. They are less sensitive to all malaria parasites but can be applied to diagnose all human attacking malaria parasites.[4,7,27] The Pfhrp2/3 mRDT targets the hrp2/3 antigens that are strictly generated by Plasmodium falciparum. Unlike the other two mRDT types, Pfhrp2/3 mRDTs are more sensitive, very stable and has very high specificity to Plasmodium falciparum. No wonder, Pfhrp2/3 mRDT is the method of choice in most rural endemic regions of Africa.[7] There have been recent reports that confirm that genetic variation of Plasmodium falciparum yields gene deletions in the Pfhr2/3 antigens hence failure of detection by the Pfhr2/3 based mRDT.[31] If local prevalence of Pfhrp2/3 deletions in the parasite populations is found to be 5% or more, there should be a change in policy towards mRDT use. This is recommended by WHO as it is the breakpoint at which there is high number of malaria positive cases that are missed out by mRDT jeopardising the use of mRDT in malaria endemic zones.[39]

This review found that there were Pfhrp2/3 deletions reported in most parts of the continent, especially in areas where Malaria is endemic. Eritrea is the first African country to report Pfhrp2/3 deletions and also had the highest prevalence. It had a change in policy direction by switching from the use of Phrp2/3 based RDTs to alternative pan based RDTs and microscopy as the principle mode of malaria diagnosis.[25] We isolated studies that had concentrated on malaria infected individuals who were either symptomatic or asymptomatic and tested positive for microscopy and/or PCR but testing negative for mRDT. Our findings did confirm the presence of the mRDT FN threat in Africa; with Eritria and Ethiopia having a very high prevalence of Pfrhp2/3 deletions, a trend that requires more scrutiny and characterization.[15,25] Geographically, the prevalence of these deletions tended to reduce as one moved Southwards of the above mentioned countries in Africa.[32,39,40]

From all the nine studies reviewed, there is a confirmation that the presence of Pfhrp2/3 gene deletions has been reported in a number of African Countries with a mean prevalence of 22.5%. In all the study findings, there is no proof or explanations for the genetic origin of the Pfhrp2/3 gene deletions. However, there is room for more studies and surveys to establish the probable origin and causative pressure that drives forward these deletions. Further, studies from many other countries on 'effect of these deletions' may provide an explanation on the origin, effect and mitigation steps to contain the negative effect of the deletions to malaria diagnosis and control.

There are hypotheses that have been advanced in an attempt to explain the origin and causes of these deletions. They include change in drug regiment from chloroquine and Sulfadoxine-Pyrimethamine (SP) to Artemisinin Combination Therapy, (ACT) and continuous use of Pfhrp2/3 RDT which may allow positive cases be thought to be negative (FN); which, if not flagged out by alternative diagnosis and treated, will proliferate and get spread to more populations.[22] There should be calls for more cross-sectional surveys in endemic regions where Pfhrp2/3 deletion status is unknown. There were limitations to the studies under review which included varied sampling procedures and types of surveys employed in the studies under review. This was a drawback to the conclusions anticipated that could influence tangible policy direction; however, these studies still gave cues to future studies.

This review also confirmed that there are reports on other factors that tend to compromise the accurate test results of mRDT. They were categorized as Parasite related (Parasite density and Prozone effect), Environmental and the end user challenges. Some mRDT manufacturer's may have directions that are not clear to the end user hence could fall below the expected accuracy standards. Other factors affecting mRDT performance may include poor environmental/storage conditions and end user professional capacities. Therefore, the service providers, the manufacturers, the quality assurance and standards teams should purpose to collaborate and cooperate in malaria diagnosis. Before the mRDTs are dispatched to the field, the quality assurance and standards officers should check and verify that correct instructions are included before packaging.

CONCLUSION

This review established reports on the presence of Pfhrp2/3 gene deletions in Africa though the prevalence was not homogenous. The methods used in data collection, sampling procedures, analysis and reporting were also varied which made reporting of the finding not consistent hence could not inform a change in policy direction. Very small samples used in some studies denied the studies and opportunity to give policy direction on the use of Pfhrpt2/3 mRDT. The prevalence of these gene deletions were significantly high in Ethiopia and Eritrea followed by Sudan, countries in the Northern hemisphere. Southern hemisphere countries recorded very low prevalences of these gene deletions. A Pfhrp2/3 gene deletion is not the only factor that compromises the mRDT performance. Other factors mentioned in the

studies under review were parasite density, the prozone phenomena, environmental factors and the mRDT end-user knowledge gap. All these tend to compromise the confidence and assurance in the mRDT outcome. This, therefore, calls for change of strategy in malaria diagnosis and treatment in endemic part of the continent. More funding on malaria-RDT research is paramount if the world is to eradicate malaria soon.

Recommendations

1. Microscopy testing and PCR should accompany mRDTs results reporting for proper surveillance of Pfhrp2/3 laboratory testing to ease up evolutionary pressure onto the Pfhrp2/3 gene mutations

2. Improve Monitoring, Evaluation and Reporting on the Impact of the deletions to malaria spread, surveillance and accurate geographical mapping of Pfhrp2/3 gene deleted cases in the country

3. Prevalence Pfhrp2/3 deletions of ≥5% in a particular zone should warrant change in policy direction towards the use of mRDT alone, to avoid FN missing out from on treatment.

4. There should be support in molecular research on the causes of the Pfhrp2/3 deletions and come up with mitigation measures to their influence towards mRDT FN results.

ACKNOWLEDGEMENT

We are grateful to all that participated directly or indirectly to the success of this study. We thank the staff of the Maseno University Zoology Department, Department of Health, Vihiga County, Kenya and Department of Public Service and Administration, Vihiga County for cooperation during data collection for this study. I particularly acknowledge all my mentors in the department for the tireless efforts in guiding this study.

ETHICAL DECLARATIONS

Reviewer Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

This study was supported by the authors.

Author Contributions

John Khamala Ongonda collected, coalesced data and drafted the manuscript under the guidance of Cyrus Ayieko from Maseno University, Kenya. Stephen Miheso (Maseno University) guided on the presentation and statistical analysis for this work.

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