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microRNAs: An opportunity to overcome significant challenges in malaria detection and control

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ABSTRACT

Organ damage and pathological disease states lead to the rapid release of microRNAs (miRNAs), a class of endogenous small non-coding RNAs, into the blood circulation. Because secreted miRNAs can be detected in biologic fluids such as plasma, they are currently being explored as promising non-invasive biomarkers of infectious and non-infectious diseases. Malaria remains a major global health challenge but still the potential of miRNAs has not been explored extensively in the context of malaria compared to other diseases. Here, we highlight important miRNAs found during different phases of the malaria life cycle in the anopheline vector and the human host. We have also put forward our opinion on how malaria parasite-stage-specific miRNAs can be incorporated into new diagnostic and prognostic tools to detect carrier mosquitoes and infected patients. In addition, we have emphasised the potential of miRNAs to be used as new therapeutics to treat severe malaria patients, an unresearched area of malaria control.

Malaria is an infectious disease that accounted for 627,000 deaths and 241 million malaria cases globally in 2020, with most deaths and cases reported in the WHO African region (WHO, 2021). Imported infections also contribute to fatal malaria cases in non-endemic countries (Zoller et al., 2009; Mischlinger et al., 2020). Five species of *Plasmodium* parasites cause disease in humans: *Plasmodium falciparum* (*Pf*), *P. vivax* (*Pv*), *P. knowlesi* (*Pk*), *P. ovale* (*Po*), and *P. malariae* (*Pm*) (Garcia, 2010). Most malaria cases are due to *Pf* and *Pv* infections (WHO, 2020). *Pf* is known to cause the majority of malarial deaths, but *Pv* infections have also been associated with life-threatening complications (Gupta et al., 2015, 2016; Anvikar et al., 2020). Clinical manifestations of malaria range from asymptomatic to uncomplicated and severe disease, depending on endemicity and age (Wassmer et al., 2015). Female *Anopheles* mosquitoes transmit *Plasmodium* parasites to humans by injecting sporozoites into the subcutaneous vasculature during their blood meals (White, 2017).

Traditionally, malaria control has predominantly relied on two approaches: effective case management and vector control (White, 2004). The former approach mainly involves the delivery of antimalarials such as chloroquine, sulfadoxine-pyrimethamine, amodiaquine, mefloquine, halofantrine, quinine, artemisinin, and artemisinin-based combination therapy (ACT). The use of ACTs in particular has played a large role in global malaria reductions. However, there is evidence of emerging artemisinin-resistant *Plasmodium* strains circulating in the WHO African region (Balikagala et al., 2021). This may lead to resistance to ACT partner drugs that has previously occurred in the Greater Mekong subregion (WHO, 2021), potentially threatening current malaria control policies (White, 2004). Similarly, increasing *pfhrp2* deletion reports and sequence variability within the *pfhrp2* (Gendrot et al., 2019; Nyataya et al., 2020) also represent a threat to malaria elimination efforts as PfHRP2 antigen-based rapid diagnostic tests are extensively used in malaria endemic countries (Verma et al., 2018).

MicroRNAs (miRNAs) are 18–24 nucleotide-long, non-coding RNAs that are rapidly released into the blood circulation upon infection and organ damage (Gupta and Wassmer, 2021). They regulate gene expression endogenously at the post-transcriptional level, either through translation repression or mRNA degradation (Gupta and Wassmer, 2021). Secreted miRNAs are extremely stable in biologic fluids, which makes them highly promising non-invasive biomarkers to detect an infection and early-stage tissue or organ damage (Zhou et al., 2016; Tribolet et al., 2020; Gupta and Wassmer, 2021). While miRNA-based biomarkers of various infectious diseases (Tribolet et al., 2020) and organ injuries (Zhou et al., 2016) have been identified, very little has been done in the context of malaria. Our published analyses of samples

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from well-characterised Mozambican children and Indian adults have shown the association of miR-3158-3p with severe falciparum malaria, highlighting for the first time a promising candidate biomarker for diagnosis across geographical regions and age groups (Gupta et al., 2021a, 2021b). Interestingly, several specific miRNAs (miR-14, 92a, 124, 275, 305, 309, and 989) have also been identified in *Pf*-infected *Anopheles* mosquitoes (Gupta and Wassmer, 2021). Thus, there is potential to incorporate parasite-stage-specific miRNAs into new diagnostic and prognostic tools to detect both carrier mosquitoes and infected patients (Gupta and Wassmer, 2021), thereby overcoming the emerging challenges associated with the loss of *pfhrp2*. Furthermore, multiplex miRNA-based assays could allow the evaluation of miRNA profiles in patients, ultimately improving diagnostic specificity and sensitivity. Their high stability in biologic fluids makes them suitable candidates for point-of-care testing, especially in limited-resource settings. However, pre-analysis factors such as different blood collection tubes, storage temperatures, a high number of freeze-thaw cycles, and miRNA isolation methods can affect miRNA levels (Sourvinou et al., 2013; Glinge et al., 2017). Fortunately, these limitations can be overcome by standardized sample collection, use of an appropriate miRNA isolation method and the immediate analysis of samples at the point of care level will circumvent any potential storage issues. In addition, incorporating exo- and endogenous controls in the miRNA analysis can ensure the robustness of data as described previously (Sourvinou et al., 2013; Glinge et al., 2017). Several miRNA-based lateral flow assays currently in development showed promising results in detecting circulating miRNAs associated with different type of cancers (Gao et al., 2014; Zheng et al., 2018; Dong et al., 2021). Despite advantages of miRNA-based tools over standard malaria diagnostic methods, only a few studies have investigated the potential of miRNAs using human samples in malaria research.

In addition to the prognostic and diagnostic potential of miRNAs, they can also pave the way for new therapeutic avenues in malaria. miRNA-based therapeutics can be divided into two categories: inhibitors of miRNAs and miRNA mimics (van Rooij and Kauppinen, 2014). Inhibitors of miRNAs, or anti-miRNAs (antimiRs), are single stranded oligonucleotides that are used to inhibit the expression of the miRNA candidate, which is overexpressed during the disease (Rupaimoole and Slack, 2017). An antimiR against miR-122 is one of the classic examples of miRNA-based therapeutics, which has reached Phase II trials for treating patients with hepatitis C virus (HCV) (Rupaimoole and Slack, 2017; Hanna et al., 2019). Miravirsen, a modified locked nucleic acid (LNA) antimiR, acts by sequestering and inhibiting miR-122 from binding to the HCV genome, thereby preventing its multiplication (Janssen et al., 2013). In contrast, miRNA mimics are synthetic, small, double-stranded RNA molecules with the same sequence as a naturally occurring miRNA. Therefore, exhausted miRNA expression that can occur during disease can be restored using a miRNA mimic. The miR-34 mimic, MRX34, has reached Phase I clinical trials for treating cancer (Rupaimoole and Slack, 2017). Similarly, miR-16-, miR-21-, miR-29-, miR-92- and miR-155-based therapeutics are also in Phase I and II clinical trials to test their efficacy in wound healing, heart failure, cancer and other conditions (Rupaimoole and Slack, 2017; Hanna et al., 2019). Based on these promising results, miRNA-modulating molecules may have great potential in malaria research and could help to overcome drug-resistant *Plasmodium* strains. Most analyses of drug-resistant malaria parasites have focused on identifying mutations and correlating with differences in the expression levels of resistance-related genes (Balikagala et al., 2021). However, alterations in the expression of regulatory miRNAs could also be responsible for influencing resistance-related gene expression, as shown in tumor cell models of drug resistance (Abdi et al., 2016) but this direction of research is yet to be explored by malaria researchers. Furthermore, studies have demonstrated that vaccines could influence miRNA levels (Drury et al., 2019; Atherton et al., 2019). The first malaria vaccine recently endorsed by the WHO for all children under five in moderate to high *Pf* transmission settings in Sub-Saharan Africa, RTS, S/AS01 (Mosquitix™), provides an opportunity to identify up- or down-regulated miRNAs associated with protection in high numbers of vaccinated children. Such miRNAs could be used to develop miRNA-based therapeutics.

To conclude, miRNAs represent a very promising tool in the fight against malaria and have the potential not only to allow the detection of parasites in mosquitoes and patients, but also to diagnose early-stage tissue or organ damage, significantly boosting clinical outcomes as well as the success of malaria control programs. In addition, the presence of several miRNA mimics and antimiRs in Phase I and II trials highlights the need for funding agencies and malaria elimination programs to invest in future research identifying potential miRNA therapeutic candidates to treat severe malaria patients.

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Ethics

No approval of the institutional review committee was needed.

CRediT authorship contribution statement

Ruhi Sikka: Methodology, Conceptualization, Writing – review & editing. **Praveen Kumar Bharti:** Methodology, Conceptualization, Writing – review & editing. **Himanshu Gupta:** Methodology, Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

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