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Biology and epidemiology of *Plasmodium falciparum* and *Plasmodium vivax* gametocyte carriage: Implication for malaria control and elimination

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ABSTRACT

Malaria is among the leading public health problems worldwide. Female anopheles mosquito orchestrates the transmission of malaria by taking gametocytes and introducing sporozoite while taking blood meals. Interrupting transmission is the major strategy for malaria elimination. The gametocyte stage is essential for the onward transmission of malaria. Thus, understanding its basic biology and epidemiology is key to malaria control and elimination. Therefore, the current review focuses on revealing the biology, prevalence, and determinants of gametocyte carriage as well as its implication on mitigation of malaria. It also illustrates the role of asymptomatic and sub-microscopic *Plasmodium* infections and G-6-PD deficiency in gametocyte carriage and hence malaria transmission.

Gametocytogenesis is initiated at committed merozoites and gives rise to the development of gametocytes. The trigger for gametocytogenesis depends on the host, parasite, and intervention factors. Gametocytes pass through five developmental stages identifiable by molecular markers. A considerable number of malaria patients carry gametocytes at a sub-microscopic level, thereby serving as a potential infectious reservoir of transmission. Factors involving the human host, *Plasmodium* parasite, and intervention parameters play a critical role in gametocyte biology and prevalence.

The contribution of asymptomatic and sub-microscopic infections to malaria transmission is unknown. The clear impact of G-6-PD deficiency on malaria control and elimination remains unclear. Lack of clarity on such issues might impede the success of interventions. Basic science and epidemiological studies should continue to overcome the challenges and cope with the ever-evolving parasite and guide interventions.

1. Introduction

Malaria is an infectious disease caused by the introduction of a *Plasmodium* parasite into the bloodstream via a female anopheline mosquito bite (World Health Organization, 2017a). *Plasmodium* infection occurs in humans and mosquitos because of sporozoite and gametocyte entry, respectively (World Health Organization, 2017a; *Encyclopedia of Malaria*, 2013; Gaur et al., 2016). Malaria remained as one of the leading causes of morbidity and mortality worldwide (World Health Organization, 2015a; World Health

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Organization, 2020). In 2020, malaria cases and deaths were estimated to be 241 million and 627 thousand, respectively. Sub-Saharan Africa bears the lion's share of the malaria burden, primarily affecting pregnant women and children under the age of five (World Health Organization, 2020). *Plasmodium falciparum (P. falciparum)* is the most lethal species, accounting for more than 90% of malaria mortality worldwide, whereas *Plasmodium vivax (P. vivax)* is the most common (World Health Organization, 2020; World Health Organization, 2021a). The devastating impact of malaria continued despite global efforts (World Health Organization, 2015a; World Health Organization, 2020).

Vector control strategies such as insecticide-treated nets (ITNs) and indoor-residual spray (IRS) have been the mainstay of malaria combat (World Health Organization, 2017a; World Health Organization, 2021b; Ariey et al., 2013). Recent advances in diagnosis, treatment, and research have raised the prospect of eliminating malaria by interrupting transmission (Gaur et al., 2016; *PLOS Medicine*, 2017a). Improved case management including combination therapy is a transmission-blocking strategy advocated to eliminate malaria (World Health Organization, 2017a; World Health Organization, 2021b; Hemingway et al., 2016). Nevertheless, the ever-growing resistance to anti-malarial drugs and insecticides forms the bottleneck to achieving elimination (World Health Organization, 2020; Ariey et al., 2013). Therefore, it is imperative to expand comprehensive efforts to find efficacious anti-malarial drugs and vaccines that interrupt the transmission- and lead to elimination (World Health Organization, 2015a; Hemingway et al., 2016; Ngwa et al., 2016).

Elimination of malaria, particularly *P. falciparum*, by interrupting transmission, is postulated in such a way that if we can clear-out infection among all human populations and render *P. falciparum* noninfectious to mosquitoes for a period longer than the lifespan of mosquito, then the transmission would stop (World Health Organization, 2017a; World Health Organization, 2015a). Even though blocking human to mosquito transmission facilitates control and elimination of *P. falciparum*, the hypnozoite stage of *P. vivax* further complicates the overall elimination work. Hypnozoites, the dormant liver-stages, constitute potentially infective parasites for a long time after the initial infection (World Health Organization, 2015b).

A transmission-blocking approach involves a combination of schizonticidal and gametocytocidal drugs that clear the asexual stage and inhibit the gametocytogenesis process, respectively (World Health Organization, 2021b; Baker, 2010). An alternative approach is the use of transmission-blocking vaccines (TBVs) including those hindering the development of gametes and/or complement-mediated destruction of gametes; developing antibodies against antigens expressed in the mosquito mid-gut; and developing antibodies against antigens expressed on the surface of gametocyte infected red blood cells (RBCs) (Gaur et al., 2016; Hemingway et al., 2016; Chawla et al., 2021). In general, the success of malaria elimination through the transmission-blocking strategy depends mainly on understanding the gametocyte stage that mediates transmission (Hemingway et al., 2016; Ngwa et al., 2016; Drakeley et al., 2006; Ngotho et al., 2019). Therefore, this review is aimed at providing an insight into the biology and epidemiology of *Plasmodium* gametocytes that can help develop tools and strategies to mitigate malaria. It also shows the role of asymptomatic and sub-microscopic infections and glucose-six-phosphate dehydrogenases (G-6-PD) deficiency on gametocyte carriage. We also raised important questions to drive research.

2. Biology of *Plasmodium* gametocyte

Malaria transmission from human to mosquito vector needs the presence of mature infectious gametocytes in the human blood (Gaur et al., 2016; Ngwa et al., 2016; Bousema and Drakeley, 2011; Wampfler et al., 2014; Koepfli and Yan, 2018a). Gametocytes of *Plasmodium* parasites are the sexual stages originating from the asexual stage in human RBCs and serve as precursors for gamete formation in the mosquito mid-gut (Meibalan and Marti, 2017; Venugopal et al., 2020). Since gametocytes play vital role in malaria transmission, their prevalence, and density serve as a proxy sign for disease transmission potential (Ariey et al., 2013; Wampfler et al., 2014; Koepfli and Yan, 2018a; Meibalan and Marti, 2017; Venugopal et al., 2020; Karl et al., 2016).

Gametocyte stages are unique to other stages of the parasite by their molecular composition and morphology. A matured gametocyte of *P. falciparum* is easily identified by its crescent-shape under a light microscope (*Encyclopedia of Malaria*, 2013; Gaur et al., 2016; Meibalan and Marti, 2017; Wampfler et al., 2013). However, it is relatively difficult to reliably identify gametocytes of other species merely by morphology. Analyzing different molecules appearing at different developmental stages helps for accurate and reliable description of gametocytes (Venugopal et al., 2020; Wampfler et al., 2013). Various proteins are uniquely expressed in microgametocyte (male) and macro-gametocyte (female) showing the specialized nature of gametocytes and the distinct roles of both gametes (Meibalan and Marti, 2017; Venugopal et al., 2020). Nearly a quarter of plasmodial genes is expressed during sexual stages. Specific RNA transcripts such as Pfs25 and Pvs25 are expressed on female gametocytes, whereas Pfs230 on both sexes (Gaur et al., 2016; Khan et al., 2005; *PLOS Medicine*, 2017b; Gebru et al., 2017; Singh, 2020).

Molecular analysis helps to differentiate male and female gametocytes starting from stage III through their level of gene expression. However, morphological identification is possible onwards from stage IV (Walzer, 2018). Macro-gametocyte has a relatively small nucleus with nucleolus and concentrated pigment. Whereas, microgametocyte has a larger nucleus with diffuse pigment devoid of the nucleolus. Sexually committed trophozoites and crescent-shaped gametocytes are often found in the blood of *P. falciparum*-infected people (Wampfler et al., 2013; *PLOS Medicine*, 2017b). Besides, surface proteins detectable by molecular tools are used to determine the species, stage, sex, and density of gametocytes (Drakeley et al., 2006; Meibalan and Marti, 2017; Wampfler et al., 2013; Joice et al., 2014; Babiker et al., 2008; Wang et al., 2020).

The development of *P. falciparum* gametocyte takes place in five stages within 10 to 12 days (Meibalan and Marti, 2017). Stage I and early stage II gametocytes are nearly circular and morphologically alike to trophozoites. The first stage of gametocyte that we can morphologically identify from trophozoites is late stage II. Late-stage III and stage IV gametocytes are elongated and spindle-shaped. Stage V is crescent shaped (falciform in Latin), hence the name *P. falciparum* (Gaur et al., 2016; Ngwa et al., 2016; Meibalan and Marti, 2017).

Early immature-stage gametocytes are sequestered in bone marrow and spleen to avoid destruction by the host immune response (Joice et al., 2014; De Niz et al., 2018). Sequestration occurs through binding between host receptors and parasite ligands (Meibalan and Marti, 2017; Venugopal et al., 2020; Joice et al., 2014; Smith, 2003; Gardiner and Trenholme, 2015). Receptors on epithelial and stromal cells of bone marrow, such as CD36, CD164, and intracellular adhesion molecule-1 enable the attachment (Gaur et al., 2016; McRobert et al., 2004). Parasite ligands involved in the attachment include *P. falciparum* erythrocyte membrane protein-1, repetitive interspersed family, and sub-telomeric variable open reading frame (Gaur et al., 2016; Smith, 2003; Gardiner and Trenholme, 2015; Petter et al., 2008; Bachmann et al., 2009; Sutherland, 2009).

Mature, stage V, gametocytes are released into circulation. Regain of deformability is speculated to prompt mature gametocytes to exit the sequestration pool and re-enter the circulation pool (Ngwa et al., 2016; Talman et al., 2004). They spend two to three days in the bloodstream until they turn infectious to mosquitoes. They also traverse skin availing themselves for ingestion into mosquitoes sucking blood (Meibalan and Marti, 2017; Talman et al., 2004).

After approximately six days of development, the mature gametocyte enters the G0 stage, which is characterized by a slowdown in genome replication, protein synthesis, and hemoglobin digestion (Baker, 2010). The mRNAs, necessary for protein synthesis during gametocyte activation in mosquito gut are prepared, but translationally inhibited. These mRNAs include Pfs16 mRNA, Pfs25 mRNA, Pfg27, Pfpeg3, Pfpeg4, Pf14744, and Pf14748 (Gaur et al., 2016; Wang et al., 2020; Sinden, 2004; Babiker and Schneider, 2008). Pfs16 mRNA located on the parasitophorous vacuole membrane, is the earliest marker of sexual stage development, and is expressed in all stages of gametocyte (Baker, 2010; Wang et al., 2020). Pfg27 is assumed responsible for a prolonged gametocyte development in *P. falciparum*. Transcription of Pfs25 mRNA starts in mature gametocytes (Gaur et al., 2016; Baker, 2010; Wang et al., 2020).

Gametocyte gets activated; and resumes genome replication and nuclear division in the mosquito mid-gut. In the mosquito mid-gut, the activated gametocytes prepare for fertilization by gametogenesis (Ngwa et al., 2016). Gametogenesis results in the formation of differentiated gametes within 20 minutes of gametocyte entry into the mosquito. Factors triggering gametogenesis include temperature decline, pH rise, and the presence of xanthurenic acid (mosquito-derived gametocyte-activating factor) (Gaur et al., 2016; Baker, 2010; Meibalan and Marti, 2017). The Pfs48/45 is a cysteine-rich surface protein essential for the fertility of male gamete (Theisen et al., 2017). During gametogenesis, gametocytes shed their RBC membrane. Male gametes then undergo three rounds of DNA replication to release up to eight motile microgametes by exflagellation. These microgametes find and fertilize a macrogamete forming a zygote that after a series of sporogonic cycle produces sporozoites to perpetuate the lifecycle (Gaur et al., 2016; Ngwa et al., 2016; Gardiner and Trenholme, 2015; Paul and Brey, 2002).

2.1. Gametocytogenesis and its triggers

Gametocytogenesis is the developmental process whereby male and female gametocytes emerge from their asexual stage precursors (Gaur et al., 2016; Baker, 2010; Talman et al., 2004). Gametocytogenesis occurs after a sexual commitment from asexual blood-stage parasites (Ngwa et al., 2016; Baker, 2010; Meibalan and Marti, 2017; Talman et al., 2004). All merozoites from a single schizont commit to follow either a sexual or asexual pathway (Talman et al., 2004; Dixon et al., 2008; Carter et al., 2013). Once committed to the sexual pathway, merozoites become exclusively either male or female gametocytes (Smith et al., 2000). Mature gametocytes can appear in the peripheral circulation within 7 to 15 days ensuing the initial wave of asexual parasites. Although it varies by species and clones, about 2% of the results of each erythrocytic schizogony cycle are gametocytes (Ngwa et al., 2016; Carter et al., 2013; Eichner et al., 2001).

Various reasons are speculated for gametocytogenesis trigger. Parasites prefer to go for transmission, as an evolutionary strategy, to take advantage by adjusting themselves with convenience of the vector (Ngwa et al., 2016; Baker, 2010; Bousema and Drakeley, 2011; Meibalan and Marti, 2017). Another hypothesis states that frequent bites from uninfected mosquitoes induce gametocytogenesis (Koepfli and Yan, 2018b). Host factors (such as immunity, hemoglobin variant, and anemia), parasite factors, and antimalarial drugs determine gametocytogenesis (World Health Organization, 2021b; Bousema and Drakeley, 2011; Meibalan and Marti, 2017; Gouagna et al., 2010; Dunyo et al., 2006). Signaling pathways may be involved in the molecular mechanism stimulating gametocytogenesis. Some of these include phorbol ester-inducing pathway, cyclic Adenosine Monophosphate signaling pathway and cholera toxin (Gaur et al., 2016; Peatey et al., 2012). Furthermore, gametocyte production in vitro increases by high parasitemia, presence of soluble factors from parasite-conditioned medium, and addition of anti-malarial drugs (Baker, 2010; Drakeley et al., 2006). These factors can also determine the gametocyte production in natural infections (Bousema and Drakeley, 2011; Meibalan and Marti, 2017).

2.1.1. Host immunity

Acquired immunity of human host affects asexual parasite densities and the resulting gametocytes. Immune responses may affect gametocytogenesis, as waves of gametocytemia usually follow uncomplicated malaria (Björkman, 2018; Sowunmi and Fateye, 2003; Bousema et al., 2010a). This suggests a potential role of the non-specific immune response, including increased tumor necrosis factor-alpha in gametocytogenesis (Bousema et al., 2010a; Nyarko and Claessens, 2021; Stevenson and Riley, 2004). Besides, studies revealed the role of specific immune response in gametocytogenesis (von Seidlein et al., 2001; Bousema et al., 2006a; Bousema, 2007). Moreover, *P. falciparum* gametocyte production in vitro has increased after addition of lymphocytes and anti-*P. falciparum* antibodies from naturally infected children in The Gambia (von Seidlein et al., 2001). These observations indeed indicate the link between immune response and gametocytogenesis (Bousema et al., 2010a; Stevenson and Riley, 2004; Bousema, 2007; Bousema et al., 2011).

2.1.2. Anemia

Various epidemiological studies described a higher prevalence of gametocytemia among anemic individuals (Ghanchi et al., 2019;

Tadesse et al., 2019; Trager, 2005). Nonetheless, these researches fail to provide a strong foundation for depicting a causal role of anemia in gametocytogenesis (von Seidlein et al., 2001; Ghanchi et al., 2019; Tadesse et al., 2019; Stepniewska et al., 2008). A larger proportion of gametocyte carriers among anemic individuals could imply a longer duration of infection that provided an extended time for gametocytogenesis (Ghanchi et al., 2019; Sowunmi et al., 2004; Barry et al., 2021). Furthermore, gametocytogenesis is triggered by the presence of reticulocytes, which are preferred cells for gametocyte development (Trager, 2005). The production of reticulocytes increases along with erythropoiesis during anemia, particularly in hemolytic anemia typical of malaria. Erythropoietin responsible for erythropoiesis has been suggested to influence gametocytogenesis and gametocyte sex ratio (Paul and Brey, 2002).

2.1.3. Parasite factors

Parasite factors such as genetic determinants trigger gametocytogenesis. A commitment of the *Plasmodium* into sexual differentiation is regulated by apicomplexan-specific transcription factor (AP2-G) (Kafsack et al., 2014; Bechtsi and AP. W., 2017; Xu et al., 2021). Regulatory proteins such as histone deacetylase 2 (PfHda2) and heterochromatin protein 1 (HP1), in turn, epigenetically control the regulatory action of *P. falciparum* AP2-G (Coleman et al., 2014). On the other hand, gametocyte development 1 (GDV1) activates AP2-G by removing HP1 (Xu et al., 2021; Filarsky et al., 2018). Studies revealed that knockdown of HP1 resulted in activation of AP2-G, and led to gametocytogenesis. These proteins are responsible for the expression and repression of AP2-G considering the existing situation (Coleman et al., 2014; Brancucci et al., 2014; Bui et al., 2021).

2.1.4. Drugs

Antimalarial drugs affect gametocytogenesis whereby some suppress, while others facilitate (Koepfli and Yan, 2018b; Dunyo et al., 2006). Antimalarial drugs that are potent against asexual stages can limit gametocyte longevity by either preventing or interrupting gametocytogenesis. The speed of gametocytogenesis disruption after treatment initiation depends upon asexual parasites clearance rate (Abdulla et al., 2016). Moreover, impact of antimalarial drugs on gametocytes depends on the type of drug, and level of drug resistance (Dunyo et al., 2006).

2.2. Plasmodium vivax gametocyte

Given its complex nature, the study of *P. vivax* gametocytes biology is limited to human infection (both natural and experimental). Its merozoites prefer reticulocytes that are rare in peripheral blood, and even takes a longer to grow on culture media rendering the in vitro study difficult (World Health Organization, 2015b; Roobsoong et al., 2015; Sinden and Gilles, 2002).

Gametocytogenesis can start with the first generation of *P. vivax* merozoites (nearly 48 hours). As a result, gametocytes can appear within three days following encounter of the first asexual parasites. These gametocytes disappear from the circulation within three days of maturation (Gaur et al., 2016; World Health Organization, 2015b; Sinden and Gilles, 2002; McKenzie et al., 2007). All developmental stages of gametocyte stay in the circulation due to absence of cyto-adherence. Infected RBCs become swollen and more flexible that can traverse through small capillaries, thus reduced chance of splenic clearance (World Health Organization, 2015b; Meibalan and Marti, 2017). The gametocyte of *P. vivax* is large and round-to-oval occupying almost all spaces of a swollen and stippled RBC. However, it is often challenging to identify the gametocyte of *P. vivax* by light microscopy (World Health Organization, 2015b).

Once mosquitoes ingest gametocytes, gametes emerge from the RBCs in the mid-gut and follow activation steps similar to *P. falciparum*. For both parasite species, sporogony takes on average nine and 16 days at 28°C and at 20°C, respectively. But, *P. vivax* can develop up to 14.5 °C than 16 °C for *P. falciparum;* partly justifying its broader geographical distribution (Meibalan and Marti, 2017; Sinden and Gilles, 2002).

3. Gametocytes and malaria transmission

In the human host, nearly 2% of the total parasite population becomes mature gametocytes that can infect the mosquito (Ngwa et al., 2016; Carter et al., 2013; Eichner et al., 2001). Gametocytes passes through a series of sexual reproduction in the mosquito before producing sporozoites ready for inoculation into a new host (Baker, 2010; Bousema and Drakeley, 2011; Meibalan and Marti, 2017; Gonçalves et al., 2017). This connotes that, the presence of gametocytes in a human host is essential for transmission, but not sufficient for infectivity (Meibalan and Marti, 2017). Therefore, the success of malaria transmission depends upon the characteristics of gametocyte, mosquito, and human host (Bousema and Drakeley, 2011; Meibalan and Marti, 2017; Gonçalves et al., 2017; Reece and Mideo, 2014).

3.1. Gametocyte infectiousness

Infectiousness of a *Plasmodium* is a function of mainly human infectious reservoir and mosquito competence (Meibalan and Marti, 2017). Human infectious reservoir refers to portion of human population capable of successfully infecting mosquitoes while harboring gametocyte (Nyarko and Claessens, 2021; World Health Organization, 2019). Gametocyte carriage and transmissibility to mosquito are parameters reflecting infectiousness. These parameters again depend on the host immunity, gametocyte sex ratio, gametocyte density, and antimalarial drugs (Meibalan and Marti, 2017; Nyarko and Claessens, 2021; Sowunmi et al., 2004; Ouédraogo et al., 2016; Schneider et al., 2007; Ouédraogo et al., 2009; Mitri et al., 2009). In most malaria cases, gametocytes exist in various densities, and can infect mosquitoes feeding on the host (Schneider et al., 2007; Ouédraogo et al., 2009; Mitri et al., 2009; Bousema et al., 2012). Yet, it is difficult to accurately measure the human infectious reservoir due to the unreliable correlation between gametocyte density and

mosquito infection rate (*PLOS Medicine*, 2017a; Koepfli and Yan, 2018a; Bousema et al., 2012).

The infectiousness of a gametocytemic individual can be confirmed using xenodiagnosis. This assay involves feeding uninfected mosquitoes on infected blood and subsequent analysis of the mosquito for infection and/or infectiousness (Ouédraogo et al., 2016; World Health Organization, 2018a; Sattabongkot et al., 2003; van der Kolk et al., 2005). Hence, mosquito-feeding assay serves as a functional analysis to determine infectiousness of an individual. Besides, this assay is a proxy indicator for detecting and quantifying oocysts or sporozoites in the mosquito (*PLOS Medicine*, 2017a; Sattabongkot et al., 2003; van der Kolk et al., 2005; Okell et al., 2008). Mosquito feeding assay can be done either by directly letting the mosquito feed on infected host (direct-feeding assay) or by in vitro feeding of the mosquito with infected blood or gametocyte culture (membrane-feeding assay) (Sattabongkot et al., 2003; van der Kolk et al., 2005; Miura et al., 2013). Despite the high efficiency of direct feeding assay, it raises ethical concern, and thus membrane-feeding assay is commonly used (Miura et al., 2013). Standard membrane-feeding assay is the gold standard for measuring transmission-reducing action of TBVs or gametocytocidal drugs (Meibalan and Marti, 2017; Bousema et al., 2012; Miura et al., 2013).

Generally, the likelihood of malaria transmission from human-to-anopheles mosquito largely depends upon density, maturity, sex composition, and circulation time of gametocyte. Besides, immune response of human and mosquito play comparable role (Meibalan and Marti, 2017; Gonçalves et al., 2017).

3.2. Determinants of gametocyte infectiousness

3.2.1. Gametocyte density

The density of both asexual and sexual stages of the parasite in blood can affect human infectiousness to mosquitoes (Meibalan and Marti, 2017; Mitri et al., 2009). Many studies reported the presence of a positive link between gametocyte density and mosquito infection rate (Schneider et al., 2007; Ouédraogo et al., 2009; Okell et al., 2012). A blood meal with a concentration below one gametocyte per liter is infectious to mosquitoes (Talman et al., 2004; Sowunmi et al., 2007). This finding also signifies the need to ensure incorporation of at least one male and one female gametocyte in a blood meal to result in mosquito infection (Ngwa et al., 2016; Meibalan and Marti, 2017; Talman et al., 2004; Reece and Mideo, 2014; Sowunmi et al., 2007). However, different scholars also reported a higher rate of infectiousness at low density. Individuals with no microscopically visible gametocyte proved infectious to mosquitoes (Nyarko and Claessens, 2021; Schneider et al., 2007; Ouédraogo et al., 2009; Okell et al., 2012). Yet, the exact mechanism behind this phenomenon remained blurred (Nyarko and Claessens, 2021; Schneider et al., 2007; Coleman et al., 2004).

On the other hand, in some studies membrane feeding experiments failed to establish correlation between the presence of gametocytes and their infectiousness (Nyarko and Claessens, 2021; Ouédraogo et al., 2009; Coleman et al., 2004; Niang et al., 2017; Whittaker et al., 2021). After their release into the circulation, *P. falciparum* gametocytes require two to three days to achieve infectiousness to mosquito, and this can partly explain the sub-standard infectious efficacy of high gametocyte density (Bousema and Drakeley, 2011; Meibalan and Marti, 2017). It can also mean the success of transmission is more about quality than quantity of the parasite (Meibalan and Marti, 2017; Gonçalves et al., 2017; Bousema et al., 2006b; Hallett et al., 2006). Generally, as per the current knowledge, neither high-density guarantee nor low-density exclude infectiousness of human to mosquito (Drakeley et al., 2006; Nyarko and Claessens, 2021; Schneider et al., 2007; Ouédraogo et al., 2009; Coleman et al., 2004).

Similarly, the correlation of *P. vivax* gametocyte density and mosquito infection is not well characterized. This is partly due to the low density parasitemia, early appearance, and disappearance of the gametocyte, and morphological complexity (World Health Organization, 2015b; Okell et al., 2012). Studies also reported infectivity at undetectable *P. vivax* gametocytemia (McKenzie et al., 2007; Bharti et al., 2006). At low gametocyte density, *P. vivax* infection is more efficient in causing mosquito infection than *P. falciparum* (Pukrittayakamee et al., 2008). *P. vivax* is quick to form gametocytes and can relapse blood-stage infections from hypnozoites. This shows *P. vivax* transmission is quicker and more persistent than *P. falciparum* (World Health Organization, 2015b).

3.2.2. Gametocyte sex ratio

Gametocyte sex ratio is one of the most important factors determining malaria transmission (Meibalan and Marti, 2017; Teboh-Ewungkem and Yuster, 2016; Reece et al., 2008). *Plasmodium* parasites lack sex chromosomes, and thus a single parasite can produce macrogamete and microgamete (Baker, 2010; Smith et al., 2000). The gametocyte sex ratio is typically female-biased; partly to balance the unduly high number of microgametes from a single microgametocyte (Paul and Brey, 2002; Stepniewska et al., 2008). It is conceived that the optimal transmission strategy is keeping the balance of microgametes and macrogametes in mosquito mid-gut (Teboh-Ewungkem and Yuster, 2016; Reece et al., 2008). Moreover, the central concern in the transmission is female-to-male gametocyte ratio that is continually adjusted in various situations (Ngwa et al., 2016; Paul and Brey, 2002; Tadesse et al., 2019; Reece and Romario, 2009).

A *P. falciparum* natural infection usually exhibits sex ratios of three or four females to one male (Talman et al., 2004; Sowunmi et al., 2007; Robert et al., 2003). Nonetheless, this ratio varies by clone, place, and season (Talman et al., 2004; Paul and Brey, 2002; Sowunmi et al., 2007; Reece and Romario, 2009; Robert et al., 2003). A longer duration of infection causes anemia due to asexual parasite proliferation. This anemia elicits erythropoiesis that triggers gametocytogenesis, mainly producing microgametocyte (Talman et al., 2004; Sowunmi et al., 2007; Robert et al., 2003).

Antimalarial drugs can affect the sex ratio by preferential clearance of gametocytes of either sex. In vitro drug tests suggest that microgametocyte is more sensitive to many antimalarial drugs than the female. In fact, anti-folates may affect males by inhibiting the folate-mediated pyrimidine synthesis required for DNA replication during exflagellation resulting in impaired fitness (Tadesse et al., 2019; Graves et al., 2012; Graves et al., 2015).

Variations in gametocyte sex ratio reflect the parasite attempt to endure sex-specific immune responses of the host. This condition

may affect the survival or infectivity of gametocytes. A male-biased ratio is preferred at lower densities of gametocytes to ensure transmission success (Paul and Brey, 2002; Reece et al., 2008; Reece and Romario, 2009). A multiplicity of infection, the presence of diverse parasite clones, shifts the ratio into a relatively even ratio to increase the likelihood of transmission of a single parasite clone (Paul and Brey, 2002; Reece and Romario, 2009).

3.2.3. Gametocyte longevity

The mean circulation time of *P. falciparum* gametocyte in the bloodstream ranges from 3.4 to 6.4 days (Ngotho et al., 2019; Eichner et al., 2001; Bousema et al., 2010b). However, an untreated infection may result in asexual parasite carriage for a long time, subsequently persisted gametocytogenesis (Ngotho et al., 2019). After termination of gametocytogenesis due to drug or immune clearance of asexual stages, the duration of gametocyte carriage is determined by considering maximum duration of gametocyte in the sequestration and circulation pools. The circulation time of gametocytes in bloodstream is affected by the natural decay of gametocytes, anti-gametocyte immunity, and gametocytocidal drugs (Bousema et al., 2010b).

Gametocytes may persist up to one month after clearance of asexual stages; by considering about 10 days and 20 days of maximum sequestration and circulation, respectively. However, drugs targeting immature gametocytes can significantly decrease the duration (Ngotho et al., 2019; Eichner et al., 2001; Nyarko and Claessens, 2021; Bousema et al., 2010b). Data on how long the naturally infected individuals carry gametocytes are limited due to logistic and ethical constraints associated with longitudinal nature of studies. A one-year longitudinal study in Liberia revealed concordant results, where 80% of the assessed 20 adults had detectable gametocytes at least once, and the longest duration of gametocyte carriage was estimated to be 188 days (Sutherland, 2009).

Several studies report that age is inversely associated with gametocyte prevalence, density, and prolonged carriage (Bousema et al., 2004; Lamptey et al., 2018; Ouédraogo et al., 2010). Young children are poor in controlling malaria due to immunity and thus carry the highest asexual parasite density (Ngwa et al., 2016). Rationally, the high asexual parasite density results in higher and longer gametocyte density and duration, respectively (Ngwa et al., 2016; Baker, 2010; Graves et al., 2012).

3.2.4. Gametocyte localization to skin compartment

Mature gametocytes in bloodstream must cross the microvasculature to reach the dermis so that mosquitoes can pick them up during a blood meal. This phenomenon is a critical component of human infectiousness and efficient transmission in general (Meibalan and Marti, 2017; Nyarko and Claessens, 2021). Studies shown a higher concentration of parasites, mainly gametocytes, in microvasculature than venous circulation (Sutherland, 2009; Bousema et al., 2012). These studies suggest that infectious mature gametocytes may preferentially localize to subdermal capillaries beneath the skin to avail themselves for pick-up by mosquitoes (Ngwa et al., 2016; Meibalan and Marti, 2017).

Although it is conceivable that infective gametocytes seek sub-dermal sites for their optimal transmission, the periodicity in gametocyte densities among venous blood samples, finger-prick blood samples, and sub-dermal capillaries lack strong evidence (Sutherland, 2009). Gametocyte concentration, according to the study in Burkina Faso, were not higher in sub-dermal skin vasculature than in other blood compartments (Barry et al., 2021).

3.2.5. Host parameters

Many studies indicated a similar contribution of the population at different age groups, while others have reported that children are more infectious at higher transmission intensities (Bousema et al., 2010a; Bousema et al., 2010b; Bousema et al., 2004; Lamptey et al., 2018; Ouédraogo et al., 2010; Zhou et al., 2016). Nevertheless, according to some studies adjusting infectivity for age-specific mosquito biting rates, adults are found to be the greater contributors to the infectious reservoir than children (Bousema et al., 2011). Additionally, adults are more likely to carry gametocytes at the borderline level of detection. Shorter sleeping hours, reduced treatment-seeking behavior, and poor adherence to treatment further explains the heightened infectiousness of adults (Ngwa et al., 2016; Bousema and Drakeley, 2011; Akim et al., 2000).

Considering settings, children could have repeated malaria infections, and often carry a higher gametocyte density (Bousema et al., 2004; Lamptey et al., 2018; Ouédraogo et al., 2010). According to a study in the high-endemic setting of Burkina Faso, that involved an age-specific mosquito-feeding assay to assess infectivity of participants to mosquitoes, children below 15 years of age contributed to much (~78%) of mosquito infections (Gonçalves et al., 2017). Similarly, the attractiveness of humans to mosquitoes depends on body size and odor. Pregnant women attract twice more mosquitoes than non-pregnant women from closer distances (Bousema and Drakeley, 2011).

3.2.6. Mosquito bite

Entomological features of anopheles mosquito, such as its blood-sampling rate, and its competency, are the principal determinants to transmission potential (Nyarko and Claessens, 2021; Gonçalves et al., 2017). As evolutionary mechanism, parasites undergo temporal fluctuation in infectiousness, which means coinciding a temporal link between peaks in infectiousness and peaks in vector biting. Hence, *Plasmodium* gametocytes should locate themselves in the right place at the right time to infect a mosquito (Ngwa et al., 2016; McKenzie et al., 2007; Hallett et al., 2006; Bharti et al., 2006).

4. Detection of *Plasmodium* gametocytes

Detecting gametocyte stages of *Plasmodium* parasite helps for diagnosis of malaria, and monitoring response to treatment (World Health Organization, 2021b; World Health Organization, 2018b). In addition to this, information on gametocyte carriage at population

level is important to characterize the transmission dynamics of malaria in a given population (World Health Organization, 2017a; Babiker et al., 2008). The laboratory diagnosis of *Plasmodium* gametocytes can be done by microscopy, molecular and serological techniques. Blood is the primary choice of sample; however, studies show the use of stool and saliva specimens to serve as potential sample for the detection (Wampfler et al., 2013; Singh et al., 2014; Kast et al., 2013).

4.1. Microscopy

Gametocytes circulate at low density, making detection difficult; particularly microscopy detects nearly 50% of gametocyte carriers (*PLOS Medicine*, 2017a). Besides, gametocytes of non-falciparum malaria species put another challenge for detection as they lack unique morphological feature (*PLOS Medicine*, 2017a; Wampfler et al., 2013; Kepple et al., 2021). According to longitudinal studies in malaria-endemic regions, microscopically detectable gametocytes are intermittently seen and disappear at some point in time of a follow-up visit. This indirectly implies that the close concurrence between gametocyte density in the circulation and the detection limit of light microscope under routine use (*PLOS Medicine*, 2017a; Okell et al., 2012; Kepple et al., 2021).

Routine microscopic examination of a 100-oil immersion field allows assessing less than 20% of the total blood of a human. Hence, it may therefore miss densities as high as 20 to 50 gametocytes/l (Koepfli and Yan, 2018b). The efficiency of microscopy can be improved by increasing the number of microscopic fields covered or concentrating parasites before examining. This was witnessed by different studies, for example, the estimates of gametocyte prevalence increased by 29% when the number of fields was changed from 100 to 200, and beyond (Wampfler et al., 2013; Koepfli and Yan, 2018b). According to some studies, the detection limit of microscopy ranges from eight to 16 gametocytes/μL of blood (Schneider et al., 2007).

Magnetic deposition microscopy (MDM) is a technique of concentrating blood-stage parasites containing hemozoin and hence allows the examination to cover a larger volume of blood thereby improving sensitivity (Zimmerman et al., 2006). Likewise, the use of MDM elevated the prevalence of *P. falciparum* gametocyte from 7.3% to 45% for symptomatic malaria in Papua New Guinea (Karl et al., 2008). Therefore, the increasing sensitivity of these methods indicates that a considerable proportion of gametocyte carriers remain hidden by solely using routine microscopy (*PLOS Medicine*, 2017a; *PLOS Medicine*, 2017b; Kepple et al., 2021; Tedla, 2019).

4.2. Molecular methods

Our understanding on prevalence and importance of low-density gametocyte carriage was renovated by introduction of RNA-based molecular tools into *Plasmodium* gametocytes detection (Gaur et al., 2016; Tedla, 2019; Cheng, 2017; Imwong et al., 2014). These tools target RNA expressed exclusively in gametocytes (*PLOS Medicine*, 2017a; *PLOS Medicine*, 2017b; Wang et al., 2020; Sinden, 2004; Tedla, 2019). The Pfs25 mRNA is chief target molecule in diagnosis of *P. falciparum* and *P. vivax* gametocyte using molecular methods. It is highly expressed in mature gametocytes (Ngwa et al., 2016). Male and female gametocytes of *P. falciparum* can be detected using specific primers for Pfs25, and Pfs230, respectively. But, in some studies both male and female gametocytes express Pfs230 (Singh, 2020). While Pfs25 and Pfg17 remain as top sensitive molecular markers to detect *P. falciparum* gametocyte (Kepple et al., 2021; Essuman, 2017). Pfs13 recently emerged as marker for microgametocyte (Santolamazza et al., 2017). Pvs25 and Pvs16 serve as major markers of *P. vivax* gametocyte (Wampfler et al., 2013). Hence, it is possible to detect and quantify gametocyte by reverse transcription PCR (RT-PCR), quantitative nucleic acid sequence-based amplification (QT-NASBA), and RT loop-mediated isothermal amplification (RT-LAMP) (*PLOS Medicine*, 2017a; Koepfli and Yan, 2018a; *PLOS Medicine*, 2017b; Babiker et al., 2008; Wang et al., 2020; Babiker and Schneider, 2008; Kepple et al., 2021; Tedla, 2019; Imwong et al., 2014).

Gametocyte-specific protein gene *Pfg377* has a similar sensitivity with Pfs25 for gametocyte detection, but it is more polymorphic (Babiker and Schneider, 2008). Single-nucleotide polymorphisms (SNP) and size differences in Pfg377 transcript allow identification of distinct gametocyte-producing parasite clones within single infections (World Health Organization, 2017a; *PLOS Medicine*, 2017a). These molecular tools improved the sensitivity up to 0.02 gametocyte/uL, despite practical and ethical challenges related to the volume of blood that can be collected and processed (Gaur et al., 2016; Tedla, 2019; Cheng, 2017; Imwong et al., 2014; Schneider et al., 2006).

Various studies witnessed the improvement of gametocyte carriage detection following introduction of molecular techniques (World Health Organization, 2017a; *PLOS Medicine*, 2017a; Tedla, 2019; Imwong et al., 2014). Moreover, the use of RT-PCR enhanced gametocyte detection by 39%-90% compared to four-26% by microscopy (*PLOS Medicine*, 2017a; Ngotho et al., 2019; Bousema et al., 2006b; Kepple et al., 2021; Schneider et al., 2006).

Serological tests can help to detect gametocytes. Major gametocyte surface antigens targeted for diagnosis of gametocytes include Pfg27 and Pfs25. However, it is important to consider that Pfg27 is found in all stages and Pfs25 is limited to matured stages only (Essangui et al., 2019). Also, Pfs230 and Pfs48/45 are also indicated as markers for exposure to gametocyte (Skinner et al., 2015).

5. Epidemiology of gametocyte carriage

5.1. Prevalence worldwide

Despite the low sensitivity of routine microscopy, substantial proportion of malaria patients carry gametocytes (*PLOS Medicine*, 2017a; Bousema et al., 2006a; Ouédraogo et al., 2016; Bousema et al., 2006b; Koepfli and Mueler, 2017; Boudová et al., 2014; Ouédraogo et al., 2007). In Papua New Guinea, population parasite prevalence of malaria and gametocyte prevalence was 6.9% and 6.1%, respectively. This figure, however, raised to 18.5% and 11.2%, respectively by RNA-based qPCR (Koepfli and Mueler, 2017).

Prevalence of *P. falciparum* and *P. vivax* gametocyte before initiation of mass screening and treatment (MST) in Indonesia were 2% and 7%, respectively (Kosasih et al., 2021). In Peruvian Amazon, gametocytes were found in 28.4% of *P. vivax* patients; besides, nearly 60% and 32% of all gametocyte carriers of *P. vivax* infection were asymptomatic and sub-microscopic, respectively (Rovira-Vallbona et al., 2017).

5.2. Prevalence in sub-Saharan Africa

A considerable proportion of malaria patients in sub-Saharan Africa carry gametocyte in their blood. These populations serve as an infectious reservoir of malaria transmission in the continent (Bousema et al., 2004; Lamptey et al., 2018; Zhou et al., 2016; Ouédraogo et al., 2007; Coalson et al., 2016; Subussa et al., 2021). At day 0, gametocytes were detected by qRT-PCR in 6% (3/48) of individuals with incident *Plasmodium* infections and 97% (58/60) of individuals with chronic infections in Burkina Faso (Ouédraogo et al., 2007). According to a longitudinal study in Mali, a sizable amount of *P. falciparum* infections, 51-89%, among the population were accompanied by gametocytemia (Adomako-Ankomah et al., 2017). Similarly, almost half of positive infections carried gametocytes, regardless of recent symptom status in Malawi (Coalson et al., 2016). In Western Kenya, 33.8% gametocyte prevalence was seen among under-five children at enrolment in the study (Bousema et al., 2004). In a longitudinal study done in Tanzania, the highest prevalence of *P. falciparum* gametocyte (30.5%) was seen on day seven of follow-up regardless of age (Akim et al., 2000).

In Sub-Saharan Africa, the prevalence of *Plasmodium* gametocyte carriage varies by the type of study population, diagnostic tool used, species of the parasite, study area and other factors (Bousema et al., 2004; Lamptey et al., 2018; Essangui et al., 2019; Boudová et al., 2014; Subussa et al., 2021). In Cameroon, *P. falciparum* mature gametocyte positivity rate was 1.9% and 8.9% by thick blood smear microscopy and Pfs25-based RT-PCR, respectively. However, in the same study, changing the diagnostic marker and tool raised the gametocyte positivity rate to 24.1% and 36.3% by RT-PCR and RT-LAMP, respectively both targeting Pfs16 (Essangui et al., 2019). Furthermore, according to a study in Blantyre (Malawi), 7.9% (27/341) of pregnant women found gametocytemic at least once during their pregnancy period (Boudová et al., 2014).

Gametocyte carriage rate in Ethiopia varies across different geographical area based on the study population, *Plasmodium* specie, season, specimen type, and the laboratory tool used. The gametocyte carriage rates of *P. falciparum* and *P. vivax* were 66.7% and 12.9% among asymptomatic *P. falciparum*- and *P. vivax*-infected individuals, respectively in Jimma town (Degefa et al., 2016). Nearly 30% of *Plasmodium* infections among pregnant women in Merti District of Oromia region carried gametocyte with the geometric mean density of 303.3 (IQR: 160-600) (Subussa et al., 2021).

A school-based study done in June and November 2015 in Amhara region, Northwestern part of Ethiopia showed varying prevalence by transmission season. In survey one from 551 students, gametocyte of *P. falciparum* and *P. vivax* was detected in 1.33% and 2.0% of the students, respectively. Whereas in survey two that involved 294 students, gametocyte of *P. falciparum* and *P. vivax* was found in 2.0% and 4.1% of the students, respectively. In this study, the overall prevalence of *P. falciparum* and *P. vivax* gametocyte was 1.54% and 2.72%, respectively. Furthermore, based on RT-PCR result, prevalence of *P. vivax* gametocyte among *P. vivax* qPCR positive individuals was 69.2% (9/13) and 57.1% (8/14) in the first and the second survey, respectively. Whereas, prevalence of *P. falciparum* gametocyte among qPCR positive individuals was 9.3% (4/43) and 10.8% (4/37) in the first and the second survey, respectively (Tadesse et al., 2017). From a study aimed to assess therapeutic efficacy of ACT against *P. falciparum* among 89 participants in Omo Nada District, Southwest Ethiopia, 12 (13.5%) and two (2.2%) of the participants carried microscopically detectable *P. falciparum* gametocytes at day zero and day 28, respectively (Mekonnen et al., 2015). Similarly, Pfs25 was detected by NASBA from capillary blood of 10 (66.7%) of 15 *P. falciparum*-positive patients in a study conducted to evaluate sensitivity of different specimens for gametocyte in Jimma, Southwest Ethiopia (Kast et al., 2013).

5.3. Gametocyte carriage in asymptomatic and sub-microscopic Plasmodium infections

Sub-microscopic parasitemia refers to a low-density blood-stage *Plasmodium* infection below the detection limit of conventional microscopy. Substantial proportion of *Plasmodium* infections are sub-microscopic and asymptomatic (World Health Organization, 2020; World Health Organization, 2021b). People with sub-microscopic and/or asymptomatic *Plasmodium* infection often exhibit poor treatment seeking behavior towards timely management of malaria. Besides, these cases often left untreated despite visiting health facilities since they test negative for conventional diagnostic techniques (Galatas et al., 2015). These circumstances provide an opportunity for the formation of gametocytes to sustain transmission (World Health Organization, 2015a). Moreover, these infections may also act as silent reservoirs that maintain low-level residual malaria transmission (Drakeley et al., 2006; Wampfler et al., 2014; Björkman and Asymptomatic, 2020).

In the progress towards malaria elimination, the accurate diagnosis and timely treatment of low density asymptomatic *Plasmodium* infections is critical since they entail gametocytes (World Health Organization, 2015a; Bousema and Drakeley, 2011). Being difficult to detect and to manage, these infections have huge implications for the design and application of anti-malarial interventions (Galatas et al., 2015; D'Alessandro, 2018).

Microscopically detectable asymptomatic *Plasmodium* infections serve as human reservoir of infection since they often persist for months and harbor gametocytes (Whittaker et al., 2021). As Rek *et al.* reported, in asymptomatic infections, gametocyte density is directly proportional to the number of infected mosquitoes (Rek et al., 2022). Although sub-microscopic gametocytemia poses less risk (Whittaker et al., 2021), as achieving malaria elimination requires targeting all the potential human reservoir of infection including them. Indeed, sub-microscopic infections constitute the major part of asymptomatic malaria (Björkman and Asymptomatic, 2020; Lin et al., 2014).

According to studies, *P. falciparum* gametocytemia is positively associated with low-density asexual parasitemia and absence of symptoms (Galatas et al., 2015; Lindblade et al., 2013). The contribution of the asymptomatic carriers to malaria transmission depends on the duration of infection, incidence of gametocyte carriage, and mosquito infectivity. Adults in malaria-endemic areas often have asymptomatic malaria, which is due in part to partial acquired immunity. (Lin et al., 2014). On the other hand, a recent study in Uganda revealed school-aged children (5-15 years old) as an important drivers of malaria transmission by contributing to about half of transmission events (Rek et al., 2022). Furthermore, pregnant women also serve considerable reservoir of malaria transmission due to their ineligibility for most of drug-based interventions (Boudová et al., 2014; Galatas et al., 2015; D'Alessandro, 2018). These population groups due to their availability to mosquito and poor treatment seeking behavior can sustain transmission of malaria in the community (Rek et al., 2022; Hailemeskel et al., 2021). Moreover, combining transmissibility and abundance in the population, asymptomatic infections were estimated to contribute to 94.7% of the infectious reservoir (Rek et al., 2022).

As majority of infections in high-transmission places are asymptomatic, it is mandatory to give them due attention (Galatas et al., 2015). On the contrary, partly due to parasite-related factor, emerging reports show the possibility of asymptomatic infection in low-transmission areas (Björkman and Asymptomatic, 2020). Therefore, asymptomatic and sub-microscopic infections with their potential to harbor gametocytes can be the silent drivers of transmission placing major challenge to control and elimination efforts (World Health Organization, 2020; Galatas et al., 2015; Lindblade et al., 2013).

5.4. Factors affecting gametocyte carriage

5.4.1. Human Host Factors

Demographic factors play important role in the process of gametogenesis that eventually determines the patterns of transmission. Hence, designing effective intervention needs clear insight into the human-infectious reservoir, and the associated risk factors affecting the likelihood of individuals contributing to mosquito infections (Bousema and Drakeley, 2011).

Concurrence of the high prevalence of G-6-PD deficiency among children aged 5-15 years was recently reported from a study done in Zambia (Kobayashi et al., 2021). The ineligibility of these population to primaquine (PQ) might have contributed for the increased prevalence (Fernando et al., 2011; Taylor, 2004).

Children and pregnant women had a higher prevalence of sub-microscopic gametocytes (39.5% and 29.7%, respectively) compared to adults (17.4%) in Ghana (Lamptey et al., 2018). Nevertheless, the proportion of gametocyte-positive infections did not show any significant association with gender in Mali (Adomako-Ankomah et al., 2017).

Pregnant women are more attractive to mosquitoes than others (Bousema and Drakeley, 2011). In a study aimed at determining the effect of IPTp-SP on gametocyte carriage among infected pregnant women in Benin, the levels of Pfs25 and Pfs230 transcripts were higher at delivery than at inclusion ($P = 0.042$). In addition, the ratio of male-to-female gametocyte transcript was higher at delivery than at inclusion ($P = 0.018$). This implies the possible role of pregnant women for increased risk of transmission (Jafari-Guemouri et al., 2018).

Age of the human host is among the important determinants of malaria prevalence and gametocyte carriage (World Health Organization, 2020; Adomako-Ankomah et al., 2017). Younger population groups carry the highest prevalence and densities of asexual malaria parasites (Bousema et al., 2004; Lamptey et al., 2018; Ouédraogo et al., 2010). In areas of high malaria transmission settings, this burden of the asexual stage often results in a high prevalence of gametocyte carriage among these groups. The role of age in immunity to gametocytogenesis is established by the better ability of the semi-immune adult host to control asexual parasite densities resulting in lower gametocyte densities. Analysis of a malaria longitudinal cohort study indicated that young age, carrying asexual parasites and recent episodes of clinical malaria increased the risk of gametocyte carriage (Muthui et al., 2019). Proportion of gametocyte-positive infections was associated with age in Mali ($P = 0.003$), and Tanzania ($P < 0.004$) (Akim et al., 2000; Adomako-Ankomah et al., 2017). Nevertheless, in low transmission intensity areas, the association between gametocytemia and age needs more study (Bousema et al., 2006a).

Adults carry much higher gametocytemia than children below the age of 12 years old, yet detection is better among children (Bousema and Drakeley, 2011; Dixon et al., 2008; Reece and Romario, 2009). Children under five years of age were 2.5 times more likely to have asymptomatic *Plasmodium* infection as compared to those 14 and above years of age. This shows the consequential likelihood of high gametocyte carriage (Degefa et al., 2016). Considering settings, children could have frequent malaria infections and carry higher gametocyte densities (Gonçalves et al., 2017; Bousema et al., 2004; Lamptey et al., 2018; Ouédraogo et al., 2010). Conversely, older children and adolescents possess the increased risk for human-mosquito transmission, despite carrying moderate gametocyte densities mainly due to their behavior, body size and relative exposure to mosquito bite. It is worth noting that this population group comprises nearly a third of the total population in sub-Saharan Africa, where malaria burden continued (Gonçalves et al., 2017).

In gametocyte-positive individuals, the density of gametocytes relative to asexual parasites increases with age. According to a study report from Burkina Faso, gametocytes take up to 2% of the total parasite population in the youngest children, but this proportion gradually increases to 15% in adults (Ouédraogo et al., 2010). In many malaria endemic settings, children have the majority of high-density parasite infections, clinical attacks, and gametocytes (Lamptey et al., 2018; Zhou et al., 2016). However, this does not necessarily mean they are the major human infectious reservoir. Adults do, due to their larger representation in the population, increased chance of sub-patent untreated infections, and a potentially higher level of commitment to the sexual pathway (Ouédraogo et al., 2010; von Seidlein et al., 2003; Grange et al., 2015). Furthermore, a study suggested that adults are responsible for 28 to 38% of mosquito infections, and this figure could rise considering the larger body size and higher exposure of adults to mosquitoes (Drakeley et al., 2000).

Furthermore, a high proportion of gametocyte carriers were observed among anemic individuals in studies from different areas (Stepniewska et al., 2008; von Seidlein et al., 2003). Likewise, anemia was associated with increased odds of gametocytemia in Kenya and Burkina Faso (Zhou et al., 2016; Sondo et al., 2021).

5.4.2. Parasite Factor

Multiple factors of the parasite determine gametocyte rate. According to the report of study conducted in Burkina Faso, the relative abundance of AP2-G ($P< 0.0001$) and gexp-5 (Correlation coefficient 0.70, $P< 0.0001$) transcripts are positively associated with gametocyte production (Ouédraogo et al., 2007).

Gametocyte carriage is strongly associated with total parasite density (Björkman, 2018; Reuling et al., 2018). A similar finding was reported from Peru, where asexual parasite density escalated the risk of *P. vivax* gametocyte by more than two-fold (95% confidence interval 1.96-2.78; $P< 0.001$) (Rovira-Vallbona et al., 2017). Individuals with low density infections than those with high-density infections harbored a fairly higher quantity of gametocytes suggesting effect of total parasite density on gametocytogenesis (Drakeley et al., 2006).

In a longitudinal study from Tanzania, high asexual parasitemia on the day of presentation ($X^2= 19.4$; $P< 0.0007$) and gametocyte positivity on the day of presentation ($X^2= 29.4$; $P< 0.001$) significantly determined the presence of gametocytes (Akim et al., 2000). Similar finding was reported from Papua New Guinea (Koepfli et al., 2015). Nevertheless, according to a study in Burkina Faso, parasite density was negatively associated with gametocyte carriage. The use of sensitive molecular tests in the study has been mentioned for the discrepancy with other studies (Koepfli and Yan, 2018b; Sondo et al., 2021). Gametocyte densities were strongly associated with total parasite densities for both *P. falciparum* (correlation coefficient= 0.83, $P= 0.010$), and *P. vivax* (correlation coefficient= 0.58, $P= 0.010$) (Tadesse et al., 2017). Keeping low-density asexual parasitemia accompanied with gametocytes is suggested as a survival strategy (Björkman, 2018; Nyarko and Claessens, 2021; Koepfli et al., 2015).

Multiclonality affects gametocyte carriage. The risk of gametocyte carriage was higher by six-fold in Ghanaian individuals infected with *P. falciparum* having both Pfmsp2 3D7 and FC27 parasite types compared with those infected with only 3D7 or FC27 parasite types (Lamptey et al., 2018). Infection with multiclonal *P. falciparum* significantly heightened ($P< 0.033$) occurrence of gametocytemia among individuals in Mali (Adomako-Ankomah et al., 2017). A similar report appeared from Burkina Faso and Papua New Guinea, where multiclonality was associated with gametocyte carriage (Ouédraogo et al., 2010; Koepfli et al., 2015). This positive association might be suggestive of infections with multiple clones that may comprise a clone capable to avoid the host immune response and eventually develop into a gametocyte (Lamptey et al., 2018; Sondo et al., 2021).

Significantly heightened ($P< 0.033$) occurrence of gametocytemia was observed among individuals with *P. falciparum* multiclonality-positive individuals in Mali (Adomako-Ankomah et al., 2017). Risk of gametocyte carriage was higher in Ghanaian individuals infected with *P. falciparum* having both Pfmsp2 3D7 and FC27 parasite types (OR= 5.92, 95% CI 1.56-22.54, $P= 0.009$) compared with those infected with only 3D7 or FC27 parasite types (Lamptey et al., 2018).

5.4.3. Intervention and other factors

Drugs often reduce the risk of gametocyte carriage either indirectly by destructing their precursors or directly by destructing gametocytes (World Health Organization, 2021b). *P. falciparum* gametocytes are susceptible to the common antimalarial drugs (Zhou et al., 2016; Gao et al., 2020). Combination therapies of malaria, such as Artemisinin-based combination therapies (ACTs), have been widely used as first-line treatment for uncomplicated malaria (World Health Organization, 2021b). They are critical in decreasing morbidity and mortality as well as blocking transmission. Treatment with artemisinin derivatives and ACTs are associated with rapid reductions in parasitemia and lower rates of gametocyte carriage,(World Health Organization, 2021c) respectively, thus a lowered likelihood of post treatment transmission of malaria (Okell et al., 2008; Bousema et al., 2006b; Portugaliza et al., 2020). The use of ACT and PQ decreased human infectiousness to mosquito up to four days after treatment (Graves et al., 2018). However, that does not guarantee a decline in gametocyte carriage, since resistance and sub-curative dose of a drug can sometimes trigger gametocytogenesis (World Health Organization, 2018a; Grange et al., 2015).

Besides, the downside of drugs is sometimes they can reach the transmission of drug-resistant malaria. The spread of chloroquine (CQ) and SP resistance may partly be associated with high gametocyte carriage after treatment and treatment failure (Dunyo et al., 2006; World Health Organization, 2018a; Barnes et al., 2008). Parasite recrudescence is associated with increased gametocyte density. A review reported that recrudescent and new infection raised the risk of gametocyte carriage by nine-fold and three-fold, respectively after day seven (Abdulla et al., 2016). A high rate of gametocytes on samples that were negative at initiation of the treatment marks the limited activity of a drug against immature gametocytes. On the other hand, the long persistence of gametocytes that were detected at initiation of the drug can be interpreted as a limited activity of the respective drug against mature gametocytes. It is conceivable that post-treatment gametocyte carriage can give a clue for the possible drug resistance (Barnes et al., 2008).

Some studies showed rise in the risk of gametocyte carriage after SP therapy (Sowunmi and Fateye, 2003; Bousema et al., 2006b). At the end of the four-week follow-up period, the proportion of gametocytemia was higher among children receiving SP (29.8%) than those not receiving the treatment (18.6%). However, the risk of gametocytemia did not significantly vary between children treated with SP and untreated children (Bousema et al., 2004). Additionally, an RCT in The Gambia showed that treatment of asymptomatic carriers of *P. falciparum* with SP did not increase risk of gametocyte carriage or density (Dunyo et al., 2006). However, studies with molecular tools suggested that this increase in microscopic gametocyte prevalence after treatment could be due to efflux of sequestered gametocytes than new gametocytogenesis (Schneider et al., 2006).

A sizeable proportion of recipients of non-ACT drugs, for example, SP and CQ, found carrying mature gametocytes after a weeklong treatment reflecting partial activity of these drugs against immature gametocytes. Also SP, CQ, amodiaquine (AQ), and quinine are

poor against mature gametocytes (Sowunmi and Fateye, 2003; Bousema et al., 2006b; Grange et al., 2015). Generally, mature gametocytes may persist up to months after successful clearance of asexual stages (Ngotho et al., 2019; Eichner et al., 2001; Okell et al., 2008; Bousema et al., 2006b).

The 8-aminoquinolines such as pamaquine, PQ, quinocide, and tafenoquine are effective against mature gametocytes (Gao et al., 2020). Primaquine is the most common and widely used 8-aminoquinoline as radical cure of *P. vivax* and as transmission-breaker for *P. falciparum* (Ngotho et al., 2019; Mitri et al., 2009; Bousema et al., 2010b; Degefa et al., 2016; Ea et al., 2014; Wampfler et al., 2017; Lin et al., 2017). Its mechanism of action is not yet meticulously understood, except its disruptive role in the metabolic function of the parasite mitochondria resulting in a reduced duration of post-treatment gametocyte carriage. Primaquine has hemotoxicity, particularly among those G-6-PD deficient individuals (World Health Organization, 2021b; Mitri et al., 2009; Bousema et al., 2010b; Ea et al., 2014). This limits the broad clinical use of PQ thereby emphasizing urgent need for safe and effective alternative drugs against mature gametocytes. Besides, PQ is not active against *P. falciparum* asexual parasites and, presumably, early gametocytes (Fernando et al., 2011; Gao et al., 2020).

An RCT from Cambodia showed the impact of a single dose PQ to bring about drop in gametocyte carriage, and thus lowered *P. falciparum* transmission. Additionally, PQ and DHP combination treatment resulted in gametocyte clearance by the end of 14 days and reduced chance to harbor gametocytes by day seven compared to those treated with DHP-alone (Lin et al., 2017). Bousema *et al.* 2016, reported similar result from systematic review and meta-analysis, in which rate of gametocytemia shrunk by use of ACT combined with either AS-MQ or AS-AO than AL-alone (Abdulla et al., 2016).

Consistent use of ITN is the profound strategy of vector control that played enormous role in reducing malaria worldwide. Besides, it is one of the recommended strategies for malaria elimination (World Health Organization, 2017a; World Health Organization, 2015a; *PLOS Medicine*, 2011). Moreover, regular use of ITN not only prevents spread of malaria, but also reduces weapons of mass dispersion- the gametocytes. In a community-based cross-sectional study in Kenya, lower odds of carrying multiple alleles of gametocyte were observed in individuals using ITN compared to their non-user counterparts (Drakeley et al., 2006; Zhou et al., 2016).

Malaria transmission follows seasonal pattern, particularly following rain suitable for mosquito breeding (Reece and Mideo, 2014). In Malawi, a higher prevalence of gametocyte carriage during rainy season (8.6%) was seen compared to dry season (3.5%) (Coalson et al., 2016). Similar finding was seen in Burkina Faso, where gametocyte density markedly raised during start and peak of the wet season. Besides, months of peak transmission not only favor gametocytogenesis, but also higher densities (Reece and Mideo, 2014). On the contrary, some results show transmission during dry season, or proportion of gametocyte-positive infections did not show any associated with seasonality in Mali. Sometimes, gametocyte carriage rises paradoxically after successfully reducing the transmission intensity by control programs (Adomako-Ankomah et al., 2017; von Seidlein et al., 2003).

Gametocyte carriage depends on geographical places. Gametocyte carriage is generally higher in high transmission areas (von Seidlein et al., 2003). Nevertheless, the investment in gametocytogenesis may be greater in low transmission settings (Drakeley et al., 2006). A compelling result in this aspect was observed from studies conducted in Kenya and Tanzania, where prevalence of parasite carriers was higher in areas with lower infectious bites per person per year than their counterpart area (Bockarie and Dagoro, 2006).

5.5. *Gametocyte carriage and glucose-six-phosphate dehydrogenase deficiency*

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, the most common human enzymopathy that is prevalent throughout malaria-endemic regions, is complicating efforts to control and eliminate malaria (World Health Organization, 2018c; Recht et al., 2018; Baird et al., 2018). This enzymopathy excludes a sizable number of people from safe and effective treatment of malaria with the only approved gametocytocidal and hypnozoiticidal drug (PQ) to *P. falciparum* and *P. vivax* infection, respectively (Baird et al., 2018). The potential of PQ to rise acute hemolytic risk in G-6-PD deficient individuals limits its extensive use as radical cure for *P. vivax* infection (World Health Organization, 2018c; Recht et al., 2018; Nguetse et al., 2016). Given the fear of dose-dependent hemolysis, the most vulnerable population groups to malaria- the pregnant women, lactating women and infants below six months of age are excluded from PQ. The erosion of PQ effectiveness in malaria endemic settings further the opportunity to gametocytemia (Baird, 2013). Limited use of PQ therapy not only increases morbidity, but also leaves behind human infectious reservoirs that sustain transmission thus delay elimination (World Health Organization, 2015b; Baird, 2013; World Health Organization: Global Malaria Programme, 2015).

On the other hand, G-6-PD deficiency prevents complicated falciparum malaria. The possible explanation behind the phenomenon is early phagocytosis of *P. falciparum* infected erythrocytes thereby lowering the risk of high parasitemia, which is among the causes of severe malaria (Kaushansky et al., 2015; Manjurano et al., 2015). With its potential for rendering uncomplicated malaria, it might contribute to asymptomatic and sub-microscopic infections. Substantial portion of these infections is missed with the conventional diagnostic methods, and therefore left untreated based on the currently recommended parasitologically confirmed malaria management algorithm. Given the mildness of the malaria, it is conceivable that it can potentially compromise the overall management of malaria by decreasing treatment-seeking behavior of patients (D'Alessandro, 2018).

Being as a bottleneck for use of PQ as radical cure for *P. vivax* infection, it might increase malaria recurrence. Moreover, it might also ensure sustained transmission of malaria (Recht et al., 2018; Baird et al., 2018). Furthermore, there is inconsistency in policy and practice on use of PQ (Recht et al., 2018). In fact, despite the lower hemolytic risks of single low dose PQ as *P. falciparum* gametocytocide use varies across countries, particularly in sub-Saharan Africa (Recht et al., 2018). The wide prevalence of G-6-PD deficiency puts a considerable block to malaria mitigation efforts by limiting PQ use (World Health Organization, 2015b; Recht et al., 2018; Assefa et al., 2018; Lo et al., 2019).

The above circumstances collectively give the parasite the edge to produce gametocyte and continue transmission. Generally, the myth of G-6-PD deficiency continued providing us with more questions than answers. Various evidences reflect the magnitude, and

impact of G-6-PD deficiency on malaria mitigation as well as the potential risk of PQ-associated harm (Recht et al., 2018; Baird et al., 2018; Howes et al., 2013). There is a need for expansion of G-6-PD testing to guide malaria interventions and continue the search for non-toxic alternatives to PQ to optimize malaria control and elimination (Howes et al., 2013).

6. Gametocytes and malaria elimination strategies

The standard control and elimination of malaria involves proper case management and interruption of transmission (World Health Organization, 2015a; Hemingway et al., 2016). The major pillars are reducing transmission from human to mosquito and vice versa through effective vector control; preventing the establishment of human infection through chemoprevention; and prompt diagnosis and treatment through universal management of cases (World Health Organization, 2015a; World Health Organization, 2021b; Ngotho et al., 2019). Thus, targeting gametocytes has become the center of attention (Talman et al., 2004; D'Alessandro, 2018; Dhiman, 2019).

6.1. Vector control

Majority of malaria control interventions such as ITN-based strategies focus at reducing transmission of malaria from human to mosquito (World Health Organization, 2017a; World Health Organization, 2021b). ITNs interrupt the transmission by protecting mosquito bite that can potentially result in either introduction of sporozoite or take-up a gametocyte. Hence, vector control is a vital strategy to control and eliminate malaria (World Health Organization, 2017a; World Health Organization, 2015a; World Health Organization, 2021b; *PLOS Medicine*, 2017b; D'Alessandro, 2018; World Health Organization, 2021d; Mueller et al., 2009).

6.2. Appropriate case management

The overall impact of treatment on malaria transmission depends on characteristics of the drug, coverage, and malaria epidemiology (World Health Organization, 2017a; World Health Organization, 2018a; Gao et al., 2020; Dhiman, 2019; McCann et al., 2020). The shift from mono-therapy to combination-therapy helped to reduce transmission intensity. In the last two decades, ACT has played a momentous role in reducing transmission and overall burden of malaria (World Health Organization, 2017a; Grueninger and Hamed, 2013). Besides, incorporation of PQ as a transmission-blocking and radical cure for *P. falciparum* and *P. vivax*, respectively is a crucial strategy of malaria control and elimination (World Health Organization, 2015a; World Health Organization, 2021b; Wampfler et al., 2017; Dhiman, 2019; Taylor, 2016; Brady et al., 2017).

6.3. Transmission-blocking chemotherapy

Transmission-blocking chemotherapy is the administration of effective antimalarial drugs to reduce transmission of gametocytes to mosquito (World Health Organization, 2017a; *PLOS Medicine*, 2017b). It is an important strategy for interrupting the transmission cycle of malaria. The WHO recommends transmission-blocking chemotherapy to eliminate *P. falciparum*. It is recommended to reduce malaria transmission in areas with low transmission, and those areas vulnerable to resistance of *P. falciparum* to artemisinin (World Health Organization, 2017a; World Health Organization, 2015a; Ariey et al., 2013; McCann et al., 2020).

Chemotherapeutic intervention aimed to interrupt transmission of malaria should consider both symptomatic and asymptomatic patients to attain its goal (World Health Organization, 2017a; *Encyclopedia of Malaria*, 2013; Ariey et al., 2013; Hemingway et al., 2016; *PLOS Medicine*, 2017b; Brady et al., 2017; Griffin et al., 2010). It involves two methods: (i) mass drug administration (MDA); and (ii) mass screening and treatment (MSAT). These methods remain a commendable tool for targeting hotspots and last foci (World Health Organization, 2021b; World Health Organization, 2015b; *PLOS Medicine*, 2017b; Grueninger and Hamed, 2013; Brady et al., 2017).

An MDA involves the time-limited distribution of antimalarial drugs to a target population with campaigns, regardless of the infection status of individuals except those to whom the drug is contraindicated (World Health Organization, 2017a; Brady et al., 2017; World Health Organization, 2017b). It allows overcoming the poor sensitivity in detecting sub-microscopic parasite densities (Brady et al., 2017; World Health Organization, 2017b). Consistent to this, MDA involving artemisinin-piperaquine (AP) and PQ shown a promising result in blocking transmission (Dicko et al., 2016; Song et al., 2010). However, due to the widespread resistance, the use of CQ and SP is recently limited (Hemingway et al., 2016). Generally, albeit its gaps, MDA helped to control and eliminate *P. falciparum* and *P. vivax* in some parts of the world (World Health Organization, 2017a; Brady et al., 2017; World Health Organization, 2016). It remains as a key element of a comprehensive malaria elimination strategy in specific settings (*PLOS Medicine*, 2017b; Brady et al., 2017; World Health Organization, 2017b).

An MSAT involves mass screening of at-risk population for the presence of malaria, regardless of symptoms, and subsequent treatment of only those found positive. The strength of this strategy is it avoids unnecessary exposure of non-infected individuals to antimalarial drugs (World Health Organization, 2017a; World Health Organization, 2021b; *PLOS Medicine*, 2017b; World Health Organization, 2016). Nonetheless, due to its dependency on the sensitivity of screening tools, MSAT may oversight sub-diagnostic carriers. It also limits people from experiencing the prophylactic advantage of a drug (Okell et al., 2009). Hence, to be successful, the MSAT approach requires an accurate, user-friendly, and low-cost diagnostic tool for all infections including asymptomatic malaria (*PLOS Medicine*, 2017b; Nyarko and Claessens, 2021; D'Alessandro, 2018; Brady et al., 2017). The use of molecular tools, such as PCR, LAMP, and QT-NASBA boosted the efficiency of MSAT (*PLOS Medicine*, 2017b; Grueninger and Hamed, 2013).

6.4. Chemoprophylaxis for Plasmodium vivax

Chemoprophylaxis is the interval-based administration of sub-therapeutic doses of antimalarial medicines that are adequate to prevent malaria disease (World Health Organization, 2015a; *PLOS Medicine*, 2017b). Preventive chemotherapy of *P. vivax* malaria can be done using CQ or PQ according to the context. CQ prophylaxis is recommended to prevent *P. vivax* malaria in pregnant women in endemic areas, where transmission is high (*PLOS Medicine*, 2017b; World Health Organization, 2016; World Health Organization: Malaria Policy Advisory Committee Meeting, 2019). In addition, a full therapeutic dose of PQ is indicated to the whole population at risk in *P. vivax* endemic settings at the elimination phase. This mass PQ preventive treatment serves as a presumptive treatment to prevent relapse, and interrupt possible transmission (World Health Organization, 2015b; *PLOS Medicine*, 2017b; Schlagenhauf and Petersen, 2013). Presumptive anti-relapse therapy is recommended for travelers who took chemoprophylaxis during travel. PART involves administration of a full therapeutic course of PQ to travelers, migrants, and others arriving malaria-free settings to avoid relapse and later transmission (World Health Organization, 2015b; *PLOS Medicine*, 2017b; World Health Organization: Malaria Policy Advisory Committee Meeting, 2019; Schlagenhauf and Petersen, 2013).

Despite its massive potentials for *P. falciparum*, targeting gametocytes is not sufficient for eliminating *P. vivax* owing to its complex biology (World Health Organization, 2015b; D'Alessandro, 2018; Baird, 2013; World Health Organization, 2016). Hypnozoite stages impose principal hurdles for elimination strategies of *P. vivax*. Currently, PQ is the only effective drug against liver-stage *P. vivax*. Yet, its wide use is limited by long therapy and hemolytic effect (Baird et al., 2018).

This calls urgent need to develop safe and effective drug against liver hypnozoites (World Health Organization, 2015a; World Health Organization, 2015b; D'Alessandro, 2018).

6.5. Transmission-blocking vaccine

Gametocyte is the major target for various tools aimed to prevent malaria transmission (Ariey et al., 2013; D'Alessandro, 2018; Sauerwein, 2007). Recent studies show a promising result on use of Pfs25, Pfs230 and Pfs48/45 gametocyte proteins as target molecules for developing a Malaria Transmission-Blocking Vaccine (MTBV) (*PLOS Medicine*, 2017b). One of these tools is the MTBV, designed to protect the public from risk of malaria by vaccinating a possibly gametocytemic individual. MTBV reduces infectiousness of gametocytes after their uptake by mosquito. Unlike to the usual vaccines, MTBV is not designed to protect vaccinated individuals directly, but to reduce malaria transmission serving as an integrated malaria control and elimination program (Reece and Romario, 2009; Robert et al., 2003; Graves et al., 2012). Generally, MTBV is vital to control, eliminate, and prevent re-introduction of malaria. It is key to prevent drug resistance from repeated use of anti-malarial drugs (Gaur et al., 2016; Ariey et al., 2013; Sauerwein, 2007).

The search for a vaccine against malaria dates back to the mid-20th century. In the beginning, the studies focused at preventing infection through immunization of mice with attenuated (irradiated) sporozoites (Gaur et al., 2016; Sauerwein, 2007). Identification of Circumsporozoite protein (CSP), the main protein for liver invasion, in the 1980s opened the door for developing a recombinant vaccine. Circumsporozoite protein is found in sporozoites and liver stages. Vaccines targeting CSP help in the induction of specific antibodies and anti-liver stage T lymphocytes. RTS, S is used as a truncated form of CSP linked to hepatitis B surface antigen (HBsAg) to produce RTS (Gaur et al., 2016; World Health Organization, 2021a).

The recent development of the RTS, S vaccine has markedly improved our chances of reaching the long-held goal of an efficient and safe vaccine against malaria (Gaur et al., 2016; Sauerwein, 2007). RTS, S/AS01 (RTS,S) is the first vaccine ever showed promising result (Hirai and Mori, 2010; Doumbo et al., 2018). It significantly reduces severe falciparum malaria, mainly among children in Africa. On a phase-based pilot in sub-Saharan Africa, the vaccine prevented severe malaria in 40% of the children, who took four doses over a four-year period (World Health Organization, 2021a). Recently WHO approved the use of RTS,S to prevent severe malaria in under-five children (World Health Organization, 2021c).

7. Conclusion and recommendation

In this review, we covered *Plasmodium* gametocyte biology, epidemiology and challenges to eliminate malaria. Understanding infectious reservoirs at community level in order to support programmatic decision-making can help optimize elimination efforts. Gametocytogenesis initiated by various triggers from host and parasite leads to gametocyte development that passes through five steps. Gametocytes continually change their location to escape host immune response, mature and optimize their traverse to mosquito. Gametocytes are essential for transmission of malaria and sustainability of the *Plasmodium* species involving two hosts. Quality and quantity of gametocyte are fundamental for the success of transmission from human to mosquitoes. Characterizing the gametocyte carriage in the human host shows the potential to infect mosquitoes.

Considerable proportion of malaria patients worldwide carry gametocyte at various quantity. Asymptomatic, sub-microscopic infections, which are often missed by the conventional malaria diagnostic tools, are potential sources of gametocyte carriage. Recurrent infections also give rise to gametocytemia and sustain malaria transmission. Hence, gametocytes are bottlenecks to elimination.

The widespread prevalence of G-6-PD deficiency confers the opportunity for gametocyte carriage by compromising the use of PQ as radical cure and transmission-blocker. The wide distribution of G-6-PD deficiency cripples malaria mitigation efforts by preventing severe disease in one hand; and favoring recurrence and gametocyte carriage on the other hand. It might act as a double-edged sword.

Previous studies, due to their cross-sectional nature, have not clearly depicted the pattern of gametocytemia and/or gametocyte carriage by modifying host, parasite, transmission setting, and seasonality. Malaria transmission potential carried by different groups

of population remains subtle.

Despite the technological advancements, the infectiousness potential of malaria-infected individuals remains vague. Majority of malaria patients with gametocyte carriage live in countries using microscope as the major diagnostic tool. We still lack a robust tool applicable for clinical purpose in diagnosis of *Plasmodium* gametocyte. This poses a big challenge to introduce targeted intervention and hence difficulty for malaria elimination.

Characterizing the spatial and temporal heterogeneity of the infectious reservoir becomes increasingly important as transmission declines if interventions are to be efficiently implemented to accelerate elimination. Unlocking gametocyte biology and its dynamics within the host is essential to develop transmission-blocking drugs and vaccines. Expansion of G-6-PD testing is important to optimize the use of PQ, particularly in malaria endemic countries. Sustained researches are critical for availability and use of non-toxic alternatives to PQ to optimize malaria control and elimination.

1. Outstanding Questions
1. How much is the contribution of sub-microscopic *Plasmodium* infection to the overall transmission of malaria?
2. What is the role of RBC polymorphisms in gametocytogenesis?
3. What is sub-microscopic gametocyte carriage rate of asymptomatic, symptomatic and sub-microscopic *P. falciparum* and *P. vivax* infections?
4. Can G-6-PD deficiency prevent severe malaria with *P. falciparum* and *P. vivax* mixed infection?
5. Does G-6-PD deficiency confer individual relief at the expense of public suffering? In this regard, can we consider G-6-PD deficiency as an opportunity-to-control or a bottleneck-to-eliminate malaria?
6. Is there a risk of drug-resistance development by *P. vivax* hypnozoite against a single-dose PQ given as gametocytocidal for *P. falciparum* during mixed infection, particularly in co-endemic areas?

Author's contributions

AA has conceived and designed this review. AA has participated in acquisition of papers and preparation of the draft manuscript. AA has critically reviewed, read and approved the manuscript.

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Availability of data and materials

All data collected for this review is included in the manuscript.

Declaration of Competing Interest

None.

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