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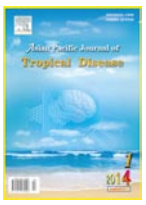

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Epidemiological aspects of vivax and falciparum malaria: global spectrum

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PEER REVIEW

Peer reviewer

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Comments

The paper bears a valuable contribution in the field of human medicine with respect to malaria considering the present scenario. The author has not only given updated information but also incorporated his own idea in this field.
The study focuses the importance of treating malaria along with developing strategies for control of vectors as effective tools for combating malaria. They should be considered together and not in isolation.
Details on Page S22

ABSTRACT

Malaria, a mosquito-borne disease, is caused by the infection of apicomplexan parasites belonging to the genus *Plasmodium*, five species of which [*Plasmodium vivax*, *Plasmodium falciparum* (*P. falciparum*), *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*] account for all forms of human malaria. *P. falciparum* is responsible for the highest degree of complications (severe malarial anaemia and cerebral malaria) and mortality in the tropics and subtropics of the world. Despite the large burden of vivax malaria, it is overlooked and left in the shadow of severity of falciparum malaria in the globe, but current reports provide evidence of severe vivax malaria symptoms similar to *P. falciparum* infection. The major challenging factor is the emergence of multidrug resistant *Plasmodium* strains to the conventionally used antimalarials over the last two decades, and, more recently, to artemisinins. The WHO recommended artemisinin based combination therapies (ACTs). The non-ACT regimens are also found to be effective, safe, and affordable compared to ACTs. However, current successful antimalarial interventions are under threat from the ability of the parasite and its mosquito vector to develop resistance to medicines and insecticides, respectively. Hence, with widespread use of effective drugs and vector control with insecticide-treated bed nets and indoor residual spraying, an ideal malaria vaccine would be the actual means of malaria prevention. This review represents the current evidence, based upon the search of SCI- and non-SCI journal, on epidemiological aspects of two forms (vivax and falciparum) of human malaria, which is still a great global concern.

KEYWORDS

Vivax malaria, Falciparum malaria, Multidrug resistance, Artemisinin based combination therapy, Malaria vaccine

1. Introduction

Human malaria, a blood infection caused by mosquito-borne apicomplexan parasites of the genus *Plasmodium*, remains a significant public health problem worldwide and is one of the leading causes of morbidity and mortality in tropical and sub-tropical regions. This parasite is a unicellular eukaryote that invades host erythrocytes and resides within a parasitophorous vacuole[1]. Actually, malaria infection starts when mosquitoes (female *Anopheles* spp.) inject sporozoites into the human skin; the parasites enter the blood stream and make their way to the liver where they develop into the exo-erythrocytic forms, which ultimately invade erythrocytes to cause symptoms. The disease can be an asymptomatic infection (in persons with accessible immunity), or a spectrum of clinical disease, ranging from mild to severe, and death in those with poor immunity. Among *Plasmodium* species causing human malaria, *Plasmodium falciparum* (*P. falciparum*) is the most virulent, and the virulence of the species has been linked to the ability of parasitized erythrocytes to adhere to the endothelial cell surface receptors expressed on blood vessel walls; this phenomenon, which is known as sequestration, allows the parasites to avoid spleen-dependent

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killing mechanisms.

The 'malignant tertian' and 'benign tertian' are two different terms that have been used traditionally for malaria caused due to the infection of *P. falciparum* and *Plasmodium vivax* (*P. vivax*), respectively, and since the terms suggest, the usual fact is that *P. falciparum* can be severe and life-threatening while *P. vivax* tends to be mild[2]. However, Genton *et al*[3] showed an association of *P. vivax* with severe malaria in Papua New Guinea, while Tjitra *et al*[4] demonstrated high morbidity and mortality associated with malaria caused by *P. vivax* infection in southern Papua, Indonesia, modifying the benign nature of vivax malaria. The *Plasmodium ovale* (*P. ovale*) and *Plasmodium malariae* (*P. malariae*) are less to cause lethality. In *P. vivax* and *P. ovale*, some of the sporozoites remains in latent condition in the liver for months to years, while, the *P. falciparum* and *P. malariae* sporozoites appear to develop just after the invasion of liver. Herein, the updated facts and phenomena related to the epidemiology of two most important forms of human malaria (vivax and falciparum), both spatial and temporal, have been depicted based upon the scientific documentation related to malaria in SCI and non-SCI journals, and in the scientific websites.

2. Etiology

Human malaria is caused by the infection of four species of protozoan parasites of the genus *Plasmodium*: *P. vivax*, *P. falciparum*, *P. ovale* and *P. malariae*; a fifth species, *Plasmodium knowlesi*, whose natural vertebrate host is *Macaca fascicularis* (macaque monkey), has currently been reported to infect humans[5-8]. *Plasmodium* belongs to the phylum Apicomplexa, a group characterized by a highly specialized apical complex, including rhoptries that play a central role in the invasion of host cells. The most of the global burden of human malaria is caused by two parasites, *P. falciparum* and *P. vivax*, among the five *Plasmodium* species known to infect humans, and *P. falciparum* causes by far the greatest morbidity and mortality, with several hundred million cases of clinical malaria and more than one million deaths occurring annually[9]. However, the sickle human haemoglobin confers a survival advantage to individuals living in endemic areas of malaria; sickle haemoglobin induces the expression of haeme oxygenase-1 that results carbon monoxide production, and help protect individual by suppressing the pathogenesis of cerebral malaria[10].

Since *P. vivax* and *P. falciparum* are the major causative agents of malaria here is a mention about the genetic diversity of the parasites, the study of which is focused on the parasites' surface proteins such as circumsporozoite protein (CSP), merozoite surface proteins: MSP-1 and MSP-2, apical membrane antigen 1 (AMA-1) and glutamate-rich protein (GLURP)[11]. Joshi[12] investigated high level of genetic diversity among *P. vivax* and *P. falciparum* strains and observed high level of length polymorphism in repeat nucleotide sequences in *P. falciparum* MSP-1, MSP-2 and GLURP, and *P. vivax* CSP, and MSP-3α. Genetic analysis using three polymorphic markers (PvAMA-1, PvCSP, PvMSP-1) for *P. vivax* and two for *P. falciparum* (PfMSP-1 and PfMSP-2) demonstrated a high degree of genetic diversity in Honduras, a low malaria transmission area[13]. Neafsey *et al*[14] reported that the global population of *P. vivax* showed greater diversity than *P. falciparum* in terms of SNP diversity, additional microsatellite and gene family variability. The microsatellite-based analysis of *P. vivax* parasites from Sri Lanka (mean genetic diversity, H_E=0.861 0), Myanmar (H_E=0.845 0), and Ethiopia (H_E=0.751 7) showed extensive genetic diversity, as has been reported by Gunawardena *et al*[15]. Kim *et al*[16] genotyped three genetic markers in *P. vivax*, *viz*., PvCSP, PvMSP 1 and PvMSP 3-α, and found a large number of distinguishable alleles: 11 for PvCSP, 35 for PvMSP 1 and 37 for PvMSP 3-α. The overall rate of mixed genotype infections was 10.6%. The PvMSP-3α and PvMSP-3β have also been used as markers in population genetic studies worldwide. In *P. vivax* four distinct allele groups: A (1.9 Kb), B (1.5 Kb), C (1.2 Kb), and D (0.3 Kb) have been detected for PvMSP-3α, while, *P. vivax* MSP-3β locus showed two allele groups: A (1.7-2.2 Kb) and B (1.4-1.5 Kb), with 5% mixed-strain infections, and in *P. falciparum*, all three known genotypes of PfMSP-1 and two of PfMSP-2 have been observed, with 24% *P. falciparum* mixed-strain infections[17]. The population-based studies of MSP-3α, MSP-3β, MSP-1, MSP-2, and other candidate antigens of *Plasmodium* species, mainly from mixed infections (*P. falciparum*+*P. vivax*), might provide information for the development of a malaria vaccine[18], and the parasitic heterogeneity might cause differences in virulence, transmissibility and responses to chemotherapy.

3. Epidemiology

The geographical distribution of endemic vivax malaria overlaps with that of endemic falciparum malaria, except in temperate zones, *viz*., the Korean peninsula, where vivax malaria only occurs[19]. Differences in *Anopheles* mosquito dynamics allow *P. vivax* transmission in temperate climates not tolerated by *P. falciparum*. In such regions, *P. vivax* infects hepatocytes but may persist as dormant hypnozoites for months or years before causing blood stage infections (relapses). *P. vivax* cannot infect Duffy-blood-group-negative reticulocytes, and is thus absent from West Africa where Duffy negativity predominates[20].

There are an estimated 550 million cases of malaria and 1 million deaths each year, and around 2.5 billion people are at risk[21], and it has been reported that about 49% of the

world's population lives in areas (109 countries in parts of Africa, Asia, the Middle East, Eastern Europe, Central and South America, Caribbean, and Oceania) where malaria is transmitted[22]. It has been recorded that among 1 700 malaria cases reported to Centers for Disease Control. In the year 2010, 65% were acquired in Africa, 19% in Asia, 15% in the Caribbean and the Americas, and <1% in Oceania[23]. Siikamaki *et al*[24] reported patients acquiring *P. vivax* and *P. falciparum* infections from different geographic regions in the globe. *P. falciparum* infections were mainly acquired in sub-Saharan Africa, and most of *P. vivax* infections on the Indian subcontinent and in Southeast Asia (Figure 1). However, it has been reported currently that each year, malaria occurs in approximately 225 million persons worldwide, and 781 000 persons, mostly African children, die from the disease[25].

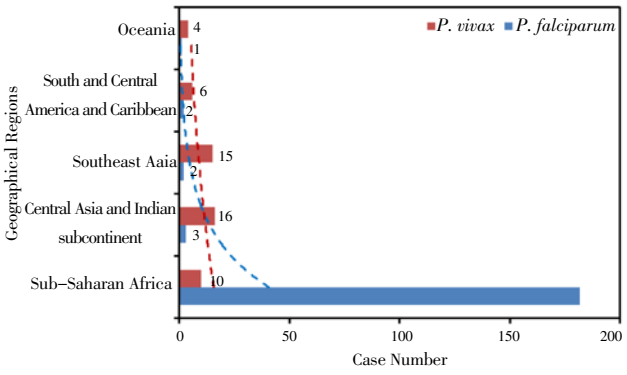

Figure 1. Vivax and falciparum malaria cases and geographic region of acquiring infection (adapted from Siikamaki *et al*., 2013)[24].

The human malaria parasite *P. vivax* is responsible for 25%-40% of the total annual cases of malaria worldwide, and it is the major cause of malaria outside Africa, mainly afflicting Asia and the Americas[26,27]. In 2005, there was an estimated 70-80 million cases of *P. vivax* infection each year, accounting for nearly 50% of all malaria cases outside Africa[28]. Mueller *et al*[19] reported that *P. vivax* is the most geographically widespread human malaria parasite causing an estimated 80-250 million global cases of vivax malaria each year. *P. falciparum* infects up to 300 million people a year resulting in the death of over 2 million annually, as reported in 2005 by Snow *et al*[29]. Children of <5 years in sub-Saharan Africa (where severe malaria caused by *P. falciparum* is the most common form of the disease) are disproportionately affected, accounting for 80% of malaria deaths worldwide[30].

The global spatial limits of *P. falciparum* malaria transmission mapping, based upon the *P. falciparum* annual parasite incidence (PfAPI), stratified the world into three areas, such as, no risk (PfAPI=0 per 1 000 pa), unstable risk (PfAPI<0.1 per 1 000 pa), and stable risk (PfAPI≥0.1 per 1 000 pa)[31,32]. The 2007 global *P. falciparum* malaria endemicity map, as has been reported by Hay *et al*[33], depicts that of the 1.38 billion people at risk of *P. falciparum* malaria, 0.69×10^8 were from Central and South East Asia, 0.66×10^8 from Africa, Yemen and Saudi Arabia, and 0.04×10^8 from the Americas. The all exposed to stable risks in the Americas were in the lowest endemicity class (*P. falciparum* parasite rate in the 2 to 10-year-old age group; $PfPR_{(2-10)}\leq5\%$). The majority (88%) of those living under stable risk in Central and South East Asia were in the low endemicity class too, while 11% were in the intermediate endemicity ($PfPR_{(2-10)}>5$ to <40%) and the remaining 1% in high endemicity ($PfPR_{(2-10)}\geq40\%$) areas. Tjitra *et al*.[4] reported clinical malaria (2004-2007) as present in 16% (60, 226/373, 450) of hospital outpatients and 32% (12, 171/37, 800) of inpatients, and among the patients with slide-confirmed malaria, 64% had *P. falciparum*, 24% *P. vivax*, and 10.5% mixed infections. Figure 2 represents the variation of infection between species.

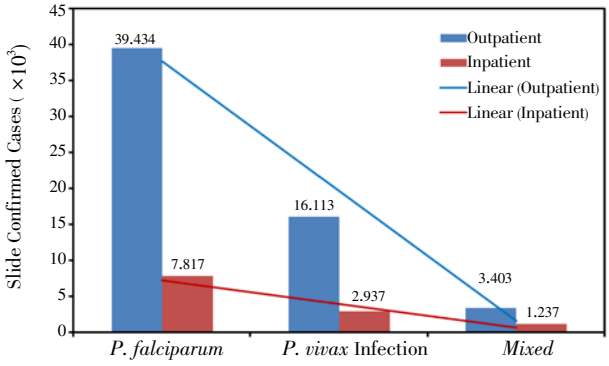

Figure 2. Symptomatic malaria patients (slide confirmed) due to *P. falciparum*, *P. vivax* and mixed infection (Tjitra *et al*., 2008)[4].

In India, *P. falciparum* is present all over the country, but its distribution is highly uneven[34,35]. It is the major cause of infection in the Northeast, Orissa, tribal settlements across the country and forests, while in the plains, *P. vivax* peak is followed by *P. falciparum* and in all other endemic areas *P. falciparum* predominates, and thrives in communities lacking awareness, resources and suffering from common poverty. The disease is endemic in the Indian state of West Bengal, accounting for 11% and 6% of the national malaria and *P. falciparum* caseloads, respectively[35], and the falciparum malaria accounted for 32% of the state malaria cases in 2004[36]. However, according to the report of National Institute Malaria Research, India, the annual incidence is of 1.5×10^6 cases, of which 40-50% is falciparum malaria[37].

Khan *et al*.[38] currently reported the high prevalence of malaria in Aligarh (India) with dominance of both *P. vivax* and *P. falciparum*, and the overall prevalence of 8.8% with maximum of 20.1% in the year 2008 and lowest of 2.3% in 2002. Thus, the combination of *P. falciparum* and *P. vivax*, different primary malaria vector species, varied ecotypes and transmission intensities have created an exigent epidemiological scenario in India[39], where there is a confliction of reports on malaria death estimation. Dhingra *et al*.[40] showed slide-positive, clinically confirmed, malaria deaths as 5 647 at all ages during 2000-2005, while WHO[41] estimated 15 000 deaths each year in India (5 000 children, 10 000 adults) caused due to malaria.

Malaria is a major travel-associated disease. The inadequate prophylaxis used for tourists and travelers in endemic areas have been the principal cause of imported malaria. According to the 2011 international travel and health book, about 125 million international travelers visit malaria endemic countries per year, and more than 10^4 cases are reported after returning home[42]. The travelers vulnerable to malaria include young children, people with chronic diseases, elderly people and pregnant women, due to their lower immune power, are at greater risk of imported malaria. The vivax malaria is frequent in travelers returning from Oceania, while the high risk of acquiring falciparum malaria is from travel to sub-Saharan Africa, South and South-East Asia, and Central and South America[43]. Many cases from Europe were registered among the travelers from Africa, South America, Asia and only a few in the Middle East[44]. Baas *et al*.[45] reported that in the Netherlands, *P. falciparum* (82%) remained the major cause of imported malaria, 94% of which was infected in sub-Saharan Africa; *P. vivax* (9.3%) the second most frequent species. However, with a mean of >4 000 cases per year during the study period, France reported the highest number of imported malaria cases; >80% of cases were caused by *P. falciparum*, the species causing almost all severe cases and death in travelers[46]. In low-transmission areas, human movement may lead to the maintenance of reservoirs of infection, complicating attempts to eliminate malaria. Importation of malaria infections might be an important contributor to maintenance of transmission even in relatively high transmission areas, as has been reported by Yukich *et al*.[47], from Ethiopia. Githeko[48] reported to originate malaria, from the travelers and immigrants, in Australia, and there is an increased risk of local malaria transmission due to current climatic factors.

The major climatic factors affecting malaria transmission and distribution include temperature, precipitation and relative humidity, and climate change is now an emerging threat to the public health when it associated with vector-borne diseases including human malaria[48,49]. Gething *et al*.[50] reported that warmer climate has an effect to increase malaria caused by the parasites *P. falciparum* and *P. vivax* in parts of Africa. The variable patterns of distribution of malaria vectors in different geographic diversity with variation in environmental conditions cause diverse in malaria endemicity, because of the developmental variability of both *P. vivax* and *P. falciparum* in mosquitoes[51,52], and hence human infection. Thus, malaria is a disease that requires involvement of humans, mosquitoes, the *plasmodium* parasites and climate.

4. Biology of the parasites

The human malaria parasites are transmitted by female mosquitoes of the genus *Anopheles*[30]; of 465 recognized species about 70 species have the capacity to disease transmission, and 41 are considered as dominant vector species/species complexes capable of transmitting malaria at the level of great concern to public health[53]. The global dominant malaria vectors map, as reported by Sinka *et al*.[54], highlight the spatial variability in the complexity of vector situation; in Africa, *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus* are co-dominant across a large part of the continent, whereas in the Asian-Pacific region there is a complex situation with multi-species coexistence and variable species dominance. There are six recognised primary vectors of malaria in India: *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles dirus*, *Anopheles fluviatilis*, *Anopheles minimus* and *Anopheles sundaicus*. The vectors of secondary importance include *Anopheles annularis*, *Anopheles varuna*, *Anopheles jeyporiensis* and *Anopheles philippinensis*.

During a blood meal, sporozoites are transmitted from the mosquito to humans and initiate infection in the liver where they reproduce industriously, and in the next stage of infection, the parasites are released from the liver cells into the bloodstream, in the form of merozoites, where they invade red blood cells (RBCs) and reproduce asexually[55].

4.1. Development of *P. falciparum*

The *P. falciparum*-infected female *Anopheles* mosquito, during a blood meal, injects sporozoite forms into the human host. These extracellular forms rapidly migrate to the liver via the bloodstream and pass through Kuppfer cells and invade hepatocytes. Each invading sporozoite divides mitotically by the process of liver schizogony, also called pre-erythrocytic schizogony or exo-erythrocytic schizogony, into thousands of liver merozoites (also called pre-erythrocytic or exo-erythrocytic merozoites)[56].

After asymptomatic hepatic infection (lasting 1 to 2 weeks), merozoites are released into the bloodstream, where they invade RBCs, and thus begin the asexual blood-stage life cycle of this parasite, called erythrocytic cycle. It has been shown that the 48-h *P. falciparum* intraerythrocytic developmental cycle initiates with merozoite invasion of erythrocyte and is followed by the formation of the parasitophorous vacuole at ring stage[57]. The *P. falciparum* subtilase, PfSUB2, one of the 3 subtilisin like serine proteases: PfSUB1, PfSUB2 and PfSUB3 of *P. falciparum*, has been reported to be responsible for the release of merozoite surface proteins during erythrocyte invasion[58].

The parasite develops through ring, trophozoite, and schizont stages, replicating to produce from 16 to 32 erythrocytic merozoites that are released during egress. In a recent analysis, merozoite production was demonstrated to range between 8 to 22 for the HB3 isolate of *P. falciparum* and 8 to 26 for the Dd2 isolate, with a median of 16 and 18, respectively[59]. Egress of *P. falciparum* involves a sudden

increase in intracellular pressure late in the blood-stage cycle, together with biochemical changes that destabilize the infected cell cytoskeleton, and these combine to promote an explosive event effectively releasing the nonmotile merozoites[60]. It has been reported that proteases are involved[61] in this process, and that this occurs in a two-step process: destruction and rupture of the internal vacuolar membrane occur distinctly and just prior to that of the erythrocyte membrane[62]. Actually, a *P. falciparum* subtilase, PfSUB1, is known to be expressed maximally in the final stages of schizont maturation[63], and the presence, in the malarial parasitophorous vacuole, of PfSUB1-mediated proteolytic processing event releases of viable parasites from the host erythrocyte[64], and in addition to PfSUB1 the cysteine protease dipeptidyl peptidase 3 also play role in the process[65]. The free merozoites are then able to invade other erythrocytes to repeat the erythrocytic cycle.

4.2. Development of P. vivax

The *P. vivax* has unique biological features[19], and the most important features distinguishing between *P. vivax* and *P. falciparum* include the development of hypnozoite, the dormant liver stage causing relapses, the formation of spherical gametocytes in the peripheral blood (gametocytes are crescentic shaped in case of *P. falciparum*), requirement of reticulocytes as host cells, in the peripheral blood, for the infection of merozoites, and presence of Schufnner's dots along the surface of infected red blood cells.

The blood stages of infection include asexual forms of the parasite that undergo repeated cycles of multiplication, and male and female gametocytes, which are nonpathogenic but are transmissible to the *Anopheles* vector, where they recombine during a brief period of diploidy and produce sporozoites[66]. The sporozoites, injected through the bite of anopheline mosquitoes, migrate to the liver within minutes, invade hepatocytes, and develop into either an actively dividing schizont, or a dormant hypnozoite, the activation of which causes the reactivation of a blood infection, clinical malaria, and the potential for transmission of the sexual gametocyte forms[19].

5. Clinical symptoms and pathogenesis

The asexual erythrocytic stage of infection is responsible for all clinical aspects of malaria, the liver stage of the infection being asymptomatic. Cycles of erythrocyte invasion, asexual reproduction by schizogony, and release of many new merozoites cause rapid parasite multiplication that gives rise to the levels of infection responsible for disease. The erythrocytic cycle varies among *Plasmodium* species: *P. falciparum*, *P. vivax*, and *P. ovale* have a cycle period of about 48 h whereas *P. malariae* has a period of about 72 h, and the simian *Plasmodium knowlesi* (which infrequently infects humans) has a 24 h period[66].

The intra-erythrocytic stages of the malaria parasite use haemoglobin from the erythrocyte cytoplasm as a food source, hydrolyzing globin to small peptides, and releasing haem, which is then converted to haemazoin[67]. As the malaria parasite, *P. falciparum*, grows within the RBCs of the human host it ingests and degrades up to 75% of host cell haemoglobin[68]. The extent of anaemia in vivax and falciparum malaria is similar (though the parasitaemia is lower in vivax than in falciparum malaria), because in case of *P. vivax* infection, nearly 34 uninfected RBCs are removed from circulation against a single infected cell while in *P. falciparum* malaria, this ratio is approximately 8:1[69].

The classic paroxysms of malaria (chills, vomiting, malaise, headache, fever and myalgia) take several asexual cycles to develop in primary infections. High fever and rigors are more common in vivax than falciparum malaria, reflecting synchronicity of schizont rupture[26]. Tjitra *et al.*[4]reported the disease severity due to *P. falciparum*, *P. vivax* and mixed infections. Figure 3 represents the variation of severity of infections between the two species.

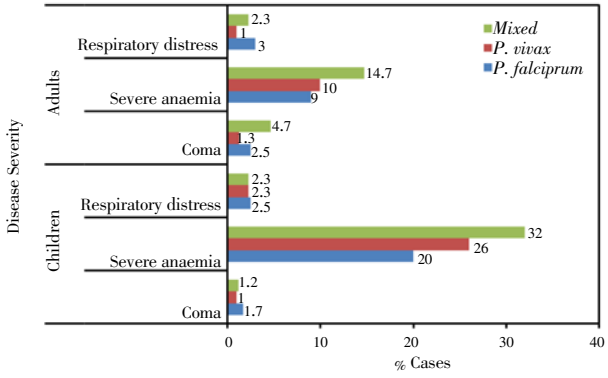

Figure 3. Spectrum of malaria illness associated with *P. falciparum*, *P. vivax* and mixed infections (Tjitra *et al.*, 2008)[4].

5.1. Falciparum malaria

The *P. falciparum* infection results 48 h lifecycle of merozoites within the erythrocytes that produces the classic symptoms of malaria. However, the unique characteristic of malaria due to *P. falciparum* infection is the sequestration of infected erythrocytes in the vasculature of various organs, particularly the brain. During this phase, erythrocytes containing *P. falciparum* mature erythrocytic stages (trophozoites and schizonts) adhere to vascular endothelium, in order to evade destruction in the spleen[70]. This cytoadherence is mediated by PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), which is expressed at the surface of *P. falciparum* infected RBCs containing mature erythrocytic stages (trophozoites and schizonts) where the protein molecules act as points of attachment to ligands upregulated in the endothelium, causing their sequestration

in the vasculature[1]. The degree of sequestration can be increased by binding of adherent infected erythrocytes to other infected erythrocytes (auto agglutination) or non-infected erythrocytes (rosetting), or by platelet mediated clumping[70,71]. PfEMP1 enables infected erythrocytes to bind to endothelial cells of host capillaries, leading to their sequestration in the peripheral vasculature and thus preventing their clearance by the host spleen. Binding of infected erythrocytes to the endothelial cells of brain capillaries causes a fatal form of malaria (cerebral malaria).

Almost all patients with cerebral malaria present with fever, rigors and/or chills, and sometimes with headache or vomiting. In cerebral malaria, *P. falciparum* is primarily attributed to sequestration of infected erythrocytes in cerebral vessels too, as mentioned above for sequestration in the peripheral vasculature. This phenomenon leads to congestion of the blood vessels and local inflammation reducing microvascular flow, and thus, neurological symptoms develop. The neurological manifestations of malaria include seizures, psychosis, agitation, impaired consciousness and coma (ending in death). The latter two are the hallmarks of cerebral malaria[70].

The normal erythrocytes are highly deformable allowing them to flow through the micro-capillaries and this property is due to their low internal viscosity, high-surface-area to volume ratio, and the elastic nature of the erythrocyte membrane and underlying cytoskeleton[72]. As the *P. falciparum* parasite grows within the erythrocyte, it loses its deformability and rigidity is increased[73], and these properties contribute to the pathogenesis of malaria, in addition to vascular adhesion of parasitised erythrocytes[72]. Mackintosh *et al.*[74] reported that in case of falciparum malaria, the metabolic demands of the proliferating parasite cells, together with the effects of massive erythrocyte lyses and ischemic damage arising from the sequestration of parasitized erythrocytes within the microvasculature, are responsible for the pathogenesis of the disease and its manifestations. Olszewski *et al.*[75] reported that systemic arginine depletion by the parasite may be a factor in human malarial hypoargininemia associated with cerebral malaria pathogenesis.

5.2. Vivax malaria

In contrast to *P. falciparum*, *P. vivax* is only capable of infecting erythrocytes, causing severe anaemia by dyserythropoiesis and destruction of infected and uninfected erythrocytes despite much lower parasitaemia[27]. *P. vivax*-infected erythrocytes become increasingly more deformable as they mature and are usually considered not to cytoadhere or sequester in the microvasculature, and these features underlie the reason why severe pathology in vivax malaria is much less common than with *P. falciparum* infection[26].

The disease due to *P. vivax* infection was thought to be clinically less severe than that associated with *P. falciparum* and rarely lethal, but studies in southeast Asia demonstrated 25% severe malaria cases due to *P. vivax*[4,76]. Although *P. vivax* malaria is regarded as a benign infection, severe and fatal complications can occur with *P. vivax* such as maternal anaemia in pregnancy and significant reduction in mean birth weight[26,77]. Moreover, drug resistance in *P. vivax* is spreading, hindering management of clinical cases, and reports of severe pathology, including respiratory distress and coma, are challenging the description of *P. vivax* malaria as 'benign'[27]. In vivax malaria, up to 10% RBCs become infected. Clinical features of malaria including anemia and its cerebral form are all associated with infected RBCs. Repeated cycles of erythrocyte invasion and rupture lead to chill, fever, headache, fatigue, other nonspecific symptoms with severe malaria, and signs of organ dysfunction [78].

Severe vivax malaria may be represented with single and multiple complications. Jaundice and haematological complication are reported as the common features followed by cerebral complication[79]. Thrombocytopaenia, acute renal, hepatic and pulmonary dysfunctions are also associated with the fever. Organ dysfunction, which is characteristic to falciparum malaria, is unusual in *P. vivax* infections, and cerebral malaria, which is caused with *P. falciparum* infection, is a presenting complication of *P. vivax* infection. Thus, the emergence of severe vivax malaria has been a newly recognized entity in different parts of the globe[80-83]. Abdallah *et al.*[84] reported the ratio of severe *P. falciparum* to severe *P. vivax* malaria as 4.3:1.0, and found that the manifestations are not significantly different between *P. falciparum* and *P. vivax* malaria. The frequencies of symptoms are depicted in Figure 4. The risk factors for severe vivax malaria include low body mass index (<20.0), high parasite count (>8×10^3/μL), age (>40 years), fever to treatment interval (>4 d), inappropriate treatment history, and other associated infection[79]. According to Genton *et al.*[3], the vivax malaria severity, compared with falciparum malaria and mixed infection, in terms of anaemia, respiratory distress and neurologic manifestation, has been represented in Figure 5.

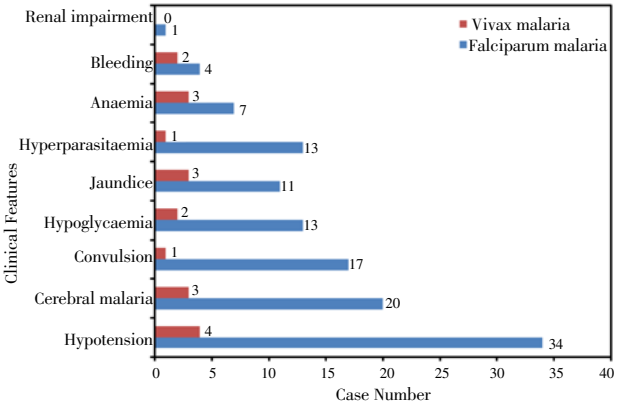

Figure 4. Clinical presentations of severe malaria (adapted from Abdallah *et al.*, 2013)[84].

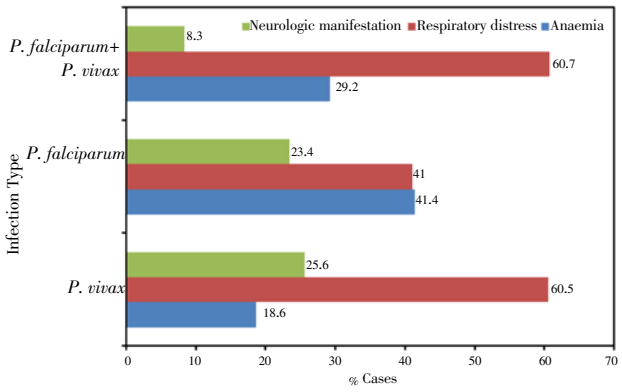

Figure 5. Severe malaria cases among children with *P. vivax*, *P. falciparum* and mixed infection (Venn diagram modified from Genton *et al*., 2008)[3].

6. Diagnosis

The traditional malaria diagnosis is based on the examination of stained blood smears under light microscope, and this method remains the gold standard for malaria diagnosis because it is inexpensive and sensitive (5-10 parasites/μL blood)[85]. However, it is labour-intensive, time-consuming, and more importantly, the lack of access to good quality microscopy services in many endemic regions limits the reliability of diagnosis.

Malaria rapid detection tests (RDTs), based on capture of the parasite antigen by mono-clonal antibodies incorporated into a test strip, provide a possibility to replace microscopic diagnosis. RDTs can be divided into two major types[86]. The first type detects histidine-rich protein 2 (HRP2), a protein uniquely synthesized by *P. falciparum* and present in the blood stream of an infected individual[87]. Some HRP2 tests are designed to also detect aldolase enzyme, a protein synthesized by all four human-infecting *Plasmodium* species[88]. The second type detects parasite lactate dehydrogenase (pLDH), an enzyme produced by human malarial parasites[85,89]. HRP-2-based RDTs are usually sensitive for the diagnosis of *P. falciparum*, although aldolase-based and parasite lactic dehydrogenase-based RDTs have suboptimal sensitivity for *P. vivax*, limiting the utility of RDTs for vivax diagnosis[26]. HRP2 tests can be less costly than the pLDH[90]. Nevertheless, many studies demonstrated that HRP2 remains in the blood stream for an extended time after successful eradication of the parasite with effective antimalarial treatment, contributing to false positives and limited specificity[91,92].

Polymerase chain reaction testing for parasite mRNA or DNA is more sensitive than microscopy[70]. It has been documented that presence of malarial retinopathy is the only clinical feature that distinguishes patients with typical histopathological features of cerebral malaria from those with alternative pathologies[70]. The loop-mediated isothermal amplification (LAMP) method is found effective in malaria diagnosis. The LAMP-Tube scanner method was found 95% sensitive and 93.3% specific in detecting *P. falciparum*, compared to the microscopy, while the sensitivity and specificity were 98.3% and 100% respectively, compared to the standard LAMP-Thermocycler[93]. The malaria LAMP, superior to expert microscopy, provides diagnostic accuracy comparable to that of nested PCR with reduced time to give result. Pakalapati *et al*.[94] evaluated microscopy, OptiMAL and multiplex PCR for the identification of *P. falciparum* and *P. vivax* from the field, and found PCR as an efficient diagnostic tool in mass screening and epidemiological purposes. The percent accuracies of the methods in terms of sensitivity, specificity and efficacy are depicted in the Figure 6.

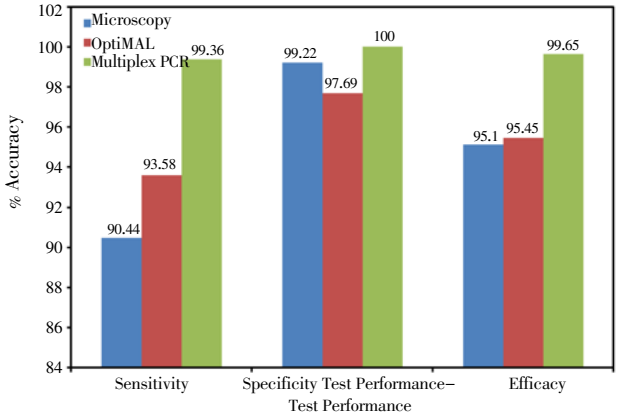

Figure 6. Test performance of three methods in the detection of *P. falciparum* and *P. vivax* (Pakalapati *et al*., 2013)[94].

7. Treatment

7.1. Conventional monotherapy

Chloroquine (CQ) is a relatively inexpensive drug for treatment of malaria[77]. The positive *P. vivax* cases should be treated with CQ in full therapeutic dose of 25 mg/kg divided over three days. The current WHO recommendations for the treatment of *P. vivax* malaria include CQ, and primaquine in case *P. vivax* is resistant to CQ[95]. Vivax malaria relapses due to the presence of hypnozoites, and for its prevention, primaquine (PQ) may be given at a dose of 0.25 mg/kg daily for 14 d under supervision, because acute intravascular haemolysis is the most serious toxic hazard of PQ, especially in people with erythrocytic glucose-6-phosphatase deficiency, and infants and pregnant women[77]. Shekalaghe *et al*.[96] reported PQ as an active agent against early as well as mature gametocytes and can substantially reduce the risk of *P. falciparum* gametocyte carriage. PQ has currently been used for prophylaxis, radical cure of vivax (and ovale) malaria, and as a single-dose gametocytocide (0.50-0.75 mg/kg) to treat falciparum malaria[97]. In falciparum malaria resistance has been detected against all currently used antimalarials: CQ, amodiaquine (AQ), mefloquine (MQ), quinine and sulfadoxine-pyrimethamine (SP) excepting the artemisinins[98,99].

7.2. Monotherapy with artemisinin and its derivatives

Artemisinin, a compound extracted from the Chinese herb, *Artemisia annua*, showed high therapeutic index in the treatment of malaria[100], and its derivatives were noted for rapid reduction of parasite biomass[101]. The unique and advantageous feature of the artemisinins includes the broad stage-specificity, against sensitive *P. falciparum* infection. Such compounds are the most potent antimalarial drugs known, reducing the parasite load by 10^5 per 48 h asexual-stage parasite cycle[102]. Artemisinin and its derivatives reduce gametocyte carriage, but they do not prevent transmission from gametocytaemia present at the time of treatment. However, reduced susceptibility of *P. falciparum* to artemisinin derivatives has been documented in the Cambodia-Thailand border region[103,104]. In the partially resistant strains of *P. falciparum*, from the Cambodia-Thailand border, the parasite load has been recorded to be reduced by 10^2 per cycle, which is an effect similar to that of slow-acting agents like quinine[102].

7.3. Artemisinin-based combination therapy

The WHO recommended artemisinin-based combination therapy (ACT) for the treatment of malaria, in countries experiencing resistance to the antimalarial agents in monotherapy, in order to counter the development of resistance in *P. falciparum* to antimalarials, and to achieve rapid resolution of parasitaemia and morbidity[105,106]. The ACT consists of an artemisinin derivative combined with long acting antimalarials like AQ, lumefantrine (L), MQ, or SP. Four combinations in priority have been recommended: artemether/L (AL), artesunate+AQ (AR+AQ), AR+MQ for low to moderate malaria transmission areas, and AR+SP in areas where SP efficacy remains high. Padmanaban *et al*.[107] reviewed on ACTs used globally in treating malaria. In the northwestern border of Thailand, introduction of AR+MQ resulted in 47% reduction in *P. falciparum* incidence[108]. In KawZulu Natal, South Africa, introduction of AL, along with vector control, reduced malaria admissions and deaths by >90%[109]. Smithuis *et al*.[110] reported that four fixed-dose WHO recommended ACTs (AR+MQ, AR+AQ, AL and dihydroartemisinin-piperaquine) had been associated with rapid parasite clearance in uncomplicated falciparum malaria, and addition of a single dose of PQ to ACT regimens has a very large additional effect on gametocytaemia[97], and therefore on malaria transmission potential, and thus the ACTs become integral to current malaria treatment strategies.

7.4. Conventional antimalarials in combination

Non-ACTs regimens of CQ, AQ and SP are reported to be effective, safe, readily available, and affordable compared to ACTs. The combination of AQ+SP was recently shown to be more effective than AL for the treatment of uncomplicated malaria[111]. In comparative trials, AQ+SP have shown similar or better efficacy than that of ACTs[111,112]. In a study[113], in Nigeria, 92% of children treated with AQ+SP showed adequate clinical and parasitological responses at Day 28 without any serious adverse reaction, and the parasite clearance time using this combination (AQ+SP) was (2.2±1.2) d, which was found similar to (2.1±0.7) d as has been reported by Sowunmi[114]. Basco *et al*.[115] showed in a comparative study of AQ and SP as monotherapy and as combination a cure rate of and 85% and 100%, respectively. It has been reported that the fall in malaria incidence started at the same time as first-line treatment with CQ has been, in Kanya, changed to treatment with SP, or SP plus CQ[116,117]. Clindamycin plus quinine is an alternative non-artemisinin-based combination recommended by World Health Organization[118].

8. Malaria vaccine

The development of malaria vaccines has been identified by different health authorities as a key component of a sustainable control programme for malaria that continues to pose a major public health threat. The malaria vaccine research has been intensified over the past few years with several vaccines tested in trials. The RTS,S vaccine has been the most promising of these candidates. The RTS,S/AS candidate malaria vaccine has been used, for immunisation of infants and children living in malaria-endemic areas in sub-Saharan Africa, with two proprietary adjuvants (AS02 and AS01) that showed promising safety profile in children and infants[119-121]. Actually, RTS,S is a recombinant antigen that consists of circumsporozoite protein (from *P. falciparum* sporozoite) fused to the hepatitis B surface antigen, and this has been formulated with two different adjuvant systems [one with an oil-in-water emulsion (AS02) and the other with liposomes (AS01)], which contain the immunostimulants MPL and QS21[122]. Asante *et al*.[123] reported that the RTS,S/AS01E candidate malaria vaccine efficacy was consistent with the target put forward by the WHO-sponsored malaria vaccine technology roadmap for a first-generation malaria vaccine. Currently, a malaria vaccine candidate (RTS,S/AS01) based on the major surface protein of the transmissible sporozoite form of the parasite advanced into phase three clinical trials[124]. The keys to the success of the vaccine are the immunogenic polymeric nature of RTS,S particles and the proprietary adjuvant AS01[125]. RTS,S/AS01E conferred sustained efficacy for at least 15 months and shows promise as a potential public health intervention against childhood malaria in malaria endemic countries, as has been documented by Olotu *et al*[126]. *P. falciparum* sporozoites (PfSPZ) are the only immunogens shown to induce such protection in humans, which is thought to be mediated by $CD8^+$ T cells in the

liver that secrete interferon-γ (IFN-γ). Epstein *et al*. report that purified irradiated PfSPZ administered was safe, but suboptimally immunogenic and protective to the prevention of infection with *P. falciparum* malaria[127].

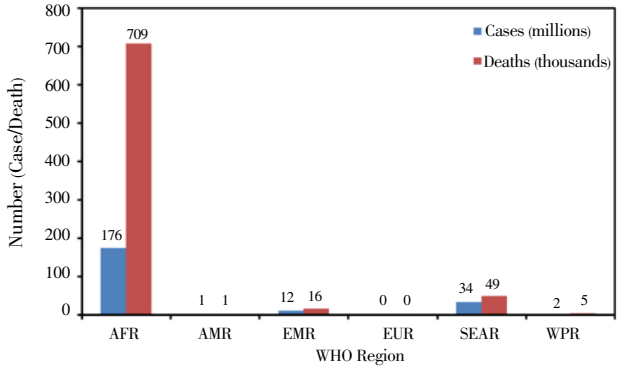

Figure 7. The world scenes of malaria mortality and morbidity in WHO regions.

AFR=Africa, AMR=Americas, EMR=Eastern Mediterranean region, EUR=Europe, SEAR=South-East Asian region, WPR=Western Pacific region (World malaria report, 2010; Text data converted)[25].

9. Prevention and control

Malaria control that lessen the disease burden to a level at which it is no longer a public health problem[106], is made possible by reduce transmission through intervention directed at either the parasite (ACTs) or the vector (ITNs or indoor residual spraying)[128]. The control relies principally on prompt and accurate diagnosis and chemotherapy with effective antimalarial drugs[129], because the prompt access to effective drugs prevents most malaria deaths at the community level, even in a context of severe malaria transmission. The use of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) of insecticides are proven preventive intervention that significantly reduce the burden of malaria. It is also imperative to provide crucial health care services, including diagnosis of malaria by RDTs and administration of ACTs, providing health education and ITNs to the individuals with malaria infection in endemic regions, and reporting morbidity and mortality statistics to health centre and the district health office[25]. Figure 7 shows the world scenes of malaria mortality and morbidity. Substantial reductions in malaria have been reported in several African countries after distribution of ITNs and the use of ACTs[130]. Roberts and Enserink[131] documented a renewed focus on elimination (cessation of local transmission of malaria within a distinct geographical region) and eradication (global disappearance of one or more species of malaria parasite). However, emerging artemisinin resistance of *P. falciparum* has been reported in some parts of the globe and pyrethroid resistance of *Anopheles* mosquitoes is increasing. Both facts representing major threats to the present malaria control strategies and the sustained effect of the approach. O'Meara *et al*.[128] reported that in some countries a decline in malaria incidence began several years before scale-up of malaria control. In other countries, the change from a failing drug CQ to a more effective drug (SP or an artemisinin combination) led to immediate improvements, and in some others the malaria reduction seemed to be associated with the scale-up of ITNs and IRS.

10. Concluding remarks

Different medications are needed to treat malaria caused by different parasites. The artemisinins have been crucial to recent successes in reducing the malaria burden, and ACTs are essential to all plans for malaria elimination. However, the ACTs (treating malaria more effectively than single antimalarial drugs and help prevnt the development of drug resistance) are found more expensive than monotherapies. In nine countries with high burden of *P. falciparum* malaria, only 16% of malaria cases were treated with ACTs in 2008[30]. Beside this, RDTs are though available at present to diagnose malaria, these remain out of reach to poor countries due to cost. Moreover, the "poor-quality antimalarial drugs lead to drug resistance and inadequate treatment, which pose an urgent threat to vulnerable populations and jeopardise progress and investments in combating malaria"[132]. Also, the spread of resistant strains causes increased treatment failure, prolonged illness, hospitalization and death, and hence additional surveillance is required to monitor the development and spread of drug resistance; alternative and improved treatments and promising malaria vaccines are in the way of development[133].

Due to the selection and spread of CQ resistance in *P. falciparum*, countries in Asia are switching to ACTs for the treatment of falciparum malaria, while CQ remains the treatment of choice for *P. vivax* malaria[77]. Thus, use of ACTs has been limited to malaria cases with *P. falciparum* infections, whereas vivax malaria patients having are mostly treated with CQ, and this separate treatment regimens is justifiable if *P. vivax* remains sensitive to CQ and diagnostic tests distinguish these two species. However, with the routine misdiagnoses and the rise and spread of CQ resistance among *P. vivax*, ACTs can be used in the treatment of both vivax and falciparum malaria in the co-endemic regions[134]. Also, a malaria vaccine, used in combination with current malaria-control tools, plays a great role in future control and hence elimination of malaria, viz., The RTS,S/AS01 vaccine provided protection against both clinical and severe malaria in African children[124].

Losing artemisinins to resistance will result in increase of malaria mortality like those that occurred during the last century when CQ failed against newly evolved drug-resistant parasites. If resistance is confined to a limited area, elimination of all *P. falciparum* parasites from the region will be the only way to prevent artemisinin resistance from spreading[135]. IRS with insecticides reduces the daily survival rate of the mosquito. ITN reduce the human biting

rate of the mosquito and its daily survival rate. In addition, the seasonal malaria chemoprevention, previously known as intermittent preventive treatment of malaria in children, is a potential tool to avert millions of malaria cases and thousands of childhood deaths in areas like Sahelian or sub-Sahelian regions of Africa (where transmission of malaria is highly seasonal), as has been reported by Cairns *et al*[136].

In view of the problems associated with antimalarial drug resistance and the use of substandard ACTs, researchers are now focusing on other alternatives, including investigation of medicinal plants known to have antiplasmodial activity[137,138], and the plant based mosquito control agents[139]. But, an ideal vaccine against malaria would be the final means of preventing the millions of cases of the disease that occur annually in our globe. Three phases in the parasite's lifecycle could effectively be targeted by host immune responses: inhibition of hepatocyte invasion at the pre-erythrocytic stage (vaccines targeting antigens such as CSP), inhibition of erythrocyte invasion stage (MSP 1 and AMA 1), and inhibition of parasite fertilization and development in the mosquito gut (oocyst/ookinete 25 kDa surface protein, Pvs25)[140,141]. However, to assist vaccine development, genetic structure and diversity of candidate antigens needs to be assessed in the *P. vivax* and *P. falciparum* populations worldwide[141]. Plus, strategies to address the problem of insecticide resistance and to tone down its effect must be successfully defined for implementation[140].

Finally, 26 of the 34 malaria-eliminating countries (76%) have a malaria burden essentially due to *P. vivax*[142], and hence *P. vivax*, which is the most common form of malaria outside Africa (infecting approximately 2.8 billion people in the globe), will be more difficult to control than the devastating *P. falciparum* infection[69], because of the latent liver-hypnozoite stage that causes multiple relapses and provides an infectious reservoir, and potentially life-threatening interaction between glucose-6-phosphate dehydrogenase deficiency (in patients) and anti-hypnozoite drug (PQ)[143]. Thus, Genton *et al*.[3] appropriately reported that interventions targeted toward *P. falciparum* only might be insufficient to eliminate the overall malaria burden, especially severe malaria, in areas where *P. falciparum* and *P. vivax* coexist, hence effective treatment regimen through proper diagnosis and vaccination are urgently needed.

Conflict of interest statement

I declare that I have no conflict of interest.

Comments

Background

Development of multi-drug resistant *Plasmodium* spp. to conventional anti-malarial drugs and the nouveau artemisinins have jeopardized effective malaria control strategy. The development of insecticide-resistant malaria vectors raises our concern. The current paper highlights the importance of the use of effective drugs together with vector control and vaccination as an effective means of combating malaria.

Research frontiers

Information in this paper clearly highlight the urgent need to develop cost-effective diagonistic tests to diagnose malaria and eradiaction of the poor quality anti-malarial drugs that increase the risks of development of drug-resistant malarial species. Development of affordable malarial drugs is the call of the hour.

Related reports

Workers from different parts of the world have reported the concern over increasing cases of drug-resistant *Plasmodium* spp. and insecticide resistant vectors. Use of artemisinins in falciparum malaria was like a boon which holds no longer true. The workers are trying their best to develop RDTs for malaria and effective ant-malarial drugs which are affordable and very much needed in the poorer parts of the world. Efforts are also being made to develop suitable malarial vaccines.

Innovations & breakthroughs

The current paper comes as a handy tool for providing detailed information on the current status of malaria and the research for combating it. The effectiveness of plant-products for malaria drugs and vector control has been heightened by the author, which are very important for futuristic research in this field.

Applications

Firstly, proper diagnosis of malaria and detection of the species of *Plasmodium* causing it is the first step for effective malaria treatment. Secondly, the tests should be affordable. Thirdly, cheap drugs should be discarded as they have already accelerated the development of drug-resistance. Fourthly, use of plant products in malaria medicine and mosquito control may serve as alternatives to artemisinins. Last but not the least, development of malaria-vaccines is required urgently.

Peer review

The paper bears a valuable contribution in the field of human medicine with respect to malaria considering the present scenario. The author has not only given updated information but also incorporated his own idea in this field.

The study focuses the importance of treating malaria along with developing strategies for control of vectors as effective tools for combating malaria. They should be considered together and not in isolation.

References

[1] Bonnefoy S, Ménard R. Deconstructing export of malaria proteins. *Cell* 2008; **134**: 20–22.

[2] Rogerson SJ, Carter R. Severe vivax malaria: newly recognised or rediscovered? *PLoS Med* 2008; **5**: e136.

[3] Genton B, D'Acremont V, Rare L, Baea K, Reeder JC, Alpers MP, et al. *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Med* 2008; **5**(6): e127.

[4] Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, et al. Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Med* 2008; **5**(6): e128.

[5] Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin Infect Dis* 2008; **46**: 165–171.

[6] Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, et al. Human infections with *Plasmodium knowlesi*, the Philippines. *Emerg Infect Dis* 2008; **14**: 811–813.

[7] White NJ. *Plasmodium knowlesi*: the fifth human malaria parasite. *Clin Infect Dis* 2008; **46**: 172–173.

[8] Vythilingam I, NoorAzian YM, Huat TC, Jiram AI, Yusri YM, Azahari AH, et al. *Plasmodium knowlesi* in humans, macaques and mosquitoes in peninsular Malaysia. *Parasit Vectors* 2008; **1**: 26.

[9] Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, Keele BF, et al. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. *Nature* 2010; **467**: 420–425.

[10] Ferreira A, Marguti I, Bechmann I, Jeney V, Chora A, Palha NR, et al. Sickle hemoglobin confers tolerance to *Plasmodium* infection. *Cell* 2011; **145**: 398–409.

[11] Arnott A, Barry AE, Reeder JC. Understanding the population genetics of *Plasmodium vivax* is essential for malaria control and elimination. *Malar J* 2012; **11**: 14.

[12] Joshi H. Markers for population genetic analysis of human plasmodia species, *P. falciparum and P. vivax*. *J Vector Borne Dis* 2003; **40**: 78–83.

[13] Lopez AC, Ortiz A, Coello J, Sosa-Ochoa W, Torres RE, Banegas EI, et al. Genetic diversity of *Plasmodium vivax* and *Plasmodium falciparum* in Honduras. *Malar J* 2012; **11**: 391.

[14] Neafsey DE, Galinsky K, Jiang RH, Young L, Sykes SM, Saif S, et al. The malaria parasite *Plasmodium vivax* exhibits greater genetic diversity than *Plasmodium falciparum*. *Nat Genet* 2012; **44**: 1046–1052.

[15] Gunawardena S, Karunaweera ND, Ferreira MU, Phone-Kyaw K, Pollack RJ, Alifrangis M, et al. Geographic structure of *Plasmodium vivax*: microsatellite analysis of parasite populations from Sri Lanka, Myanmar, and Ethiopia. *Am J Trop Med Hyg* 2010; **82**: 235–242.

[16] Kim JR, Imwong M, Nandy A, Chotivanich K, Nontprasert A, Tonomsing N, et al. Genetic diversity of *Plasmodium vivax* in Kolkata, India. *Malar J* 2006; **5**: 71.

[17] Khatoon L, Baliraine FN, Bonizzoni M, Malik SA, Yan G. Genetic structure of *Plasmodium vivax* and *Plasmodium falciparum* in the Bannu district of Pakistan. *Malar J* 2010; **9**: 112.

[18] Khatoon L, Khan IU, Shah SA, Jan MI, Ullah F, Malik SA. Genetic diversity of *Plasmodium vivax* and *Plasmodium falciparum* in Kohat District, Pakistan. *Braz J Infect Dis* 2012; **16**: 184–187.

[19] Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, et al. Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. *Lancet Infect Dis* 2009; **9**: 555–566.

[20] Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmodium vivax* in blacks: the Duffyblood-group genotype, FyFy. *N Engl J Med* 1976; **295**: 302–304.

[21] Richards JS, Beeson JG. The future for blood-stage vaccines against malaria. *Immunol Cell Biol* 2009; **87**: 377–390.

[22] Mali S, Steele S, Slutsker L, Arguin PM. Malaria Surveillance—United States, 2007. *Morbidity Mortality Weekly Rep* 2009; **58**: 1–16.

[23] Centers for Disease Control and Prevention. Malaria. Atlanta: CDC; 2013. [Online] Available from: wwwnc.cdc.gov/travel/yellowBookCh4-Malaria.aspx/. [Accessed on September 20th 2013]

[24] Siikamäki H, Kivelä P, Lyytikäinen O, Kantele A. Imported malaria in Finland 2003–2011: prospective nationwide data with rechecked background information. *Malar J* 2013; **12**: 93.

[25] World Health Organization. *World malaria report 2010*. Geneva: WHO; 2010.

[26] Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. *Am J Trop Med Hyg* 2007; **77**: 79–87.

[27] Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzi H, Caler E, et al. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature* 2008; **455**: 757–763.

[28] Guerra CA, Snow RW, Hay SI. Mapping the global extent of malaria in 2005. *Trends Parasitol* 2006; **22**: 353–358.

[29] Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005; **434**: 214–217.

[30] World Health Organization. *World malaria report 2009*. Geneva: WHO; 2009, p. 66.

[31] Guerra CA, Gikandi PW, Tatem AJ, Noor AM, Smith DL, Hay SI, et al. The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. *PLoS Med* 2008; doi:10.1371/journal.pmed.0050038.

[32] Hay SI, Smith DL, Snow RW. Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infect Dis* 2008; **8**: 369–378.

[33] Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, Noor AM, et al. A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Med* 2009; doi: 10.1371/journal.pmed.1000048.

[34] Gupta S, Gunter JT, Novak RJ, Regens JL. Patterns of *Plasmodium vivax* and *Plasmodium falciparum* malaria underscore importance of data collection from private health care facilities in India. *Malar J* 2009; **8**: 227.

[35] Kumar A, Valecha N, Jain T, Dash AP. Burden of malaria in India: retrospective and prospective view. *Am J Trop Med Hyg* 2007; **77**: 69–78.

[36] Mali S, Kachur SP, Arguin PM. Malaria surveillance—United States, 2010. *Surveillance Summaries* 2012; **61**: 1–17.

[37] Dash AP. *A profile of national institute of malaria research*. New Delhi, India: National Institute of Malaria Research; 2009.

[38] Khan HM, Shujatullah F, Ashfaq M, Raza A. Changing trends in prevalence of different *Plasmodium* species with dominance of *Plasmodium falciparum* malaria infection in Aligarh (India). *Asian Pac J Trop Med* 2011; **4**: 64–66.

[39] Shah NK, Dhillon GP, Dash AP, Arora U, Meshnick SR, Valecha N.

Antimalarial drug resistance of *Plasmodium falciparum* in India: changes over time and space. *Lancet Infect Dis* 2011; **11**: 57-64.
[40] Dhingra N, Jha P, Sharma VP, Cohen AA, Jotkar RM, Rodriguez PS, et al. Adult and child malaria mortality in India: a nationally representative mortality survey. *Lancet* 2010; **376**: 1768-1774.
[41] World Health Organization. *World malaria report 2008*. Geneva: WHO; 2008.
[42] World Health Organization. International travel and health. Geneva: WHO; 2012. [Online] Available from: http://www.who.int/ith/en/. [Accessed on September 20th 2013]
[43] Jacquerioz FA, Croft AM. Drugs for preventing malaria in travellers. *Cochrane Database Syst Rev* 2009; doi: 10.1002/14651858.CD006491.pub2.
[44] Halichidis S. Imported malaria. *J Trop Dis* 2013; **1**: 2.
[45] Baas MC, Wetsteyn JC, van Gool T. Patterns of imported malaria at the academic medical center, Amsterdam, the Netherlands. *J Travel Med* 2006; **13**: 2-7.
[46] Seringe E, Thellier M, Fontanet A, Legros F, Bouchaud O, Ancelle T, et al. Severe imported *Plasmodium falciparum* malaria, France, 1996-2003. *Emerg Infect Dis* 2011; **17**: 807- 813.
[47] Yukich JO, Taylor C, Eisele TP, Reithinger R, Nauhassenay H, Berhane Y, et al. Travel history and malaria infection risk in a low-transmission setting in Ethiopia: a case control study. *Malar J* 2013; **12**: 33.
[48] Githeko AK. Malaria and climate change. In: Alleyne G, editor. *Commonwealth health ministers' update*. London, UK: Commonwealth Secretariat; 2009, p. 40-43.
[49] Caminade C, Jones A, Morse A. The impact of climate change on malaria distribution in Africa: a multi-model approach. Liverpoor, USA: University of Liverpoor; 2012. [Online] Available from: http://www.liv.ac.uk/media/livacuk/qweci/QWeCI,presentation,Cyril,Caminade.pdf. [Accessed on September 20th 2013]
[50] Gething PW, Smith DL, Patil AP, Tatem AJ, Snow RW, Hay SI. Climate change and the global malaria recession. *Nature* 2010; **465**: 342-345.
[51] Dhiman RC, Pahwa S, Dash AP. Climate change and malaria in India: Interplay between temperatures and mosquitoes. *Regional Health Forum* 2008; **12**: 27-31.
[52] An G. Influence of climate on malaria in China. *Penn McNair Res J* 2011; **3**: 1-24.
[53] Hay SI, Sinka ME, Okara RM, Kabaria CW, Mbithi PM, Tago CC, et al. Developing global maps of the dominant *Anopheles* vectors of human malaria. *PLoS Med* 2010; doi: 10.1371/journal.pmed.1000209.
[54] Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, et al. A global map of dominant malaria vectors. *Parasit Vectors* 2012; **5**: 69.
[55] Aly AS, Vaughan AM, Kappe SH. Malaria parasite development in the mosquito and infection of the mammalian host. *Annu Rev Microbiol* 2009; **63**: 195-221.
[56] Cowman AF, Crabb BS. Invasion of red blood cells by malaria parasites. *Cell* 2006; **124**: 755-766.
[57] Bozdech Z, Llinás M, Pulliam BL, Wong ED, Zhu J, DeRis JL. The transcriptome of the intraerythrocytic developmental cycle of *Plasmodium falciparum*. *PLoS Biol* 2003; doi: 10.1371/journal.pbio.0000005.
[58] Harris PK, Yeoh S, Dluzewski AS, O'Donnel RA, Withers-Martinez C, Hackett F, et al. Molecular identification of a malaria merozoite surface sheddase. *PLoS Pathog* 2005; **1**: 241-251.
[59] Reilly HB, Wang H, Steuter JA, Marx AM, Ferdig MT. Quantitative dissection of clone-specific growth rates in cultured malaria parasites. *Int J Parasitol* 2007; **37**: 1599-1607.
[60] Glushakova S, Yin D, Li T, Zimmerberg J. Membrane transformation during malaria parasite release from human red blood cells. *Curr Biol* 2005; **15**: 1645-1650.
[61] Salmon BL, Oksman A, Goldberg DE. Malaria parasite exit from the host erythrocyte: a two-step process requiring extraerythrocytic proteolysis. *Proc Natl Acad Sci USA* 2001; **98**: 271-276.
[62] Wickham ME, Culvenor JG, Cowman AF. Selective inhibition of a two-step egress of malaria parasites from the host erythrocyte. *J Biol Chem* 2003; **278**: 37658-37663.
[63] Blackman MJ, Fujioka H, Stafford WH, Sajid M, Clough B, Fleck SL, et al. A subtilisin-like protein in secretory organelles of *Plasmodium falciparum* merozoites. *J Biol Chem* 1998; **273**: 23398-23409.
[64] Yeoh S, O'Donnell RA, Koussis K, Dluzewski AR, Ansell KH, Osborne SA, et al. Subcellular discharge of a serine protease mediates release of invasive malaria parasites from host erythrocytes. *Cell* 2007; **131**: 1072-1083.
[65] Arastu-Kapur S, Ponder EL, Fonović UP, Yeoh S, Yuan F, Fonović M, et al. Identification of proteases that regulate erythrocyte rupture by the malaria parasite *Plasmodium falciparum*. *Nat Chem Biol* 2008; **4**: 203-213.
[66] Aravind L, Iyer LM, Wellems TE, Miller LH. *Plasmodium* Biology: genomic gleanings. *Cell* 2003; **115**: 771-785.
[67] Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 2002; **419**: 498-511.
[68] Loria P, Miller S, Foley M, Tilley L. Inhibition of the peroxidative degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. *Biochem J* 1999; **339**: 363-370.
[69] Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ, et al. The anaemia of *Plasmodium vivax* malaria. *Malar J* 2012; **11**: 135.
[70] Mishra SK, Newton CR. Diagnosis and management of the neurological complications of falciparum malaria. *Nat Rev Neurol* 2009; **5**: 189-198.
[71] Zaki SA, Shanbag P. Atypical manifestations of malaria. *Res Rep Trop Med* 2011: **2**: 9-22.
[72] Maier AG, Rug M, O'Neill MT, Brown M, Chakravorty S, Szestak T, et al. Exported proteins required for virulence and rigidity of *Plasmodium falciparum*-infected human erythrocytes. *Cell* 2008; **134**: 48-61.
[73] Cooke BM, Mohandas N, Coppel RL. Malaria and the red blood cell membrane. *Semin Hematol* 2004; **41**: 173-188.
[74] Mackintosh CL, Beeson JG, Marsh K. Clinical features and pathogenesis of severe malaria. *Trends Parasitol* 2004; **20**: 597-603.
[75] Olszewski KL, Morrisey JM, Wilinski D, Burns JM, Vaidya AB, Rabinowitz JD, et al. Host-parasite interactions revealed by *Plasmodium falciparum* metabolomics. *Cell Host Microbe* 2009; **5**: 191-199.
[76] Price RN, Douglas NM, Anstey NM. New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. *Curr Opin Infect Dis* 2009; **22**: 430-435.
[77] Naing C, Aung K, Win DK, Wah MJ. Efficacy and safety of chloroquine for treatment in patients with uncomplicated *Plasmodium vivax* infections in endemic countries. *Trans R Soc*

Trop Med Hyg 2010; **104**: 695-705.
[78] Rosenthal PJ. Artesunate for the treatment of severe falciparum malaria. *N Engl J Med* 2008; **358**: 1829-1836.
[79] Mohapatra MK, Dash LK, Mohapatra A. Severe vivax malaria: a study on its clinical manifestations, risk factors, outcome and therapeutic efficacy of artesunate. *Int J Clin Case Rep* 2013; **3**: 17-25.
[80] Sarkar D, Ray S, Saha M, Chakraborty A, Talukdar A. Clinico-laboratory profile of severe *Plasmodium vivax* malaria in a tertiary care centre in Kolkata. *Trop Parasitol* 2013; **3**: 53-57.
[81] Patel MP, Kute VB, Gumber MR, Gera DN, Shah PR, Patel HV, et al. *Plasmodium vivax* malaria presenting as hemolytic uremic syndrome. *Indian J Nephrol* 2013; **23**: 74-75.
[82] Andrade BB, Reis-Filho A, Souza-Neto SM, Clarêncio J, Camargo LM, Barral A, et al. Severe *Plasmodium vivax* malaria exhibits marked inflammatory imbalance. *Malar J* 2010; **9**: 13.
[83] Mehmood A, Ejaz K, Ahmed T. Severity of *Plasmodium vivax* malaria in Karachi: a cross sectional study. *J Infect Dev Ctries* 2012; **6**: 664-670.
[84] Abdallah TM, Abdeen MT, Ahmed IS, Hamdan HZ, Magzoub M, Adam I. Severe *Plasmodium falciparum* and *Plasmodium vivax* malaria among adults at Kassala hospital, eastern Sudan. *Malar J* 2013; 12: 148.
[85] Moody AH, Chiodini PL. Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria pLDH antigen detection. *Br J Biomed Sci* 2002; **59**: 228-231.
[86] Ochola LB, Vounatsou P, Smith T, Mabaso ML, Newton CR. The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. *Lancet Infect Dis* 2006; **6**: 582-588.
[87] Howard RJ, Uni S, Aikawa M, Aley SB, Leech JH, Lew AM, et al. Secretion of a malarial histidine-rich protein (Pf HRP II) from *Plasmodium falciparum*-infected erythrocytes. *J Cell Biol* 1986; **103**: 1269-1277.
[88] Lee N, Baker J, Bell D, McCarthy J, Cheng Q. Assessing the genetic diversity of the aldolase genes of *Plasmodium falciparum* and *Plasmodium vivax* and its potential effect on performance of aldolase-detecting rapid diagnostic tests. *J Clin Microbiol* 2006; **4**: 4547-4549.
[89] Palmer CJ, Bonilla JA, Bruckner DA, Barnett ED, Miller NS, Haseeb MA, et al. Multicenter study to evaluate the OptiMAL test for rapid diagnosis of malaria in U.S. hospitals. *J Clin Microbiol* 2003; **41**: 5178-5182.
[90] Chaijaroenkul W, Wongchai T, Ruangweerayut R, Na-Bangchang K. Evaluation of rapid diagnostics for *Plasmodium falciparum* and *P. vivax* in Mae Sot malaria endemic area, Thailand. *Korean J Parasitol* 2011; **49**: 33-38.
[91] Bell DR, Wilson DW, Martin LB. False-positive results of a *Plasmodium falciparum* histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. *Am J Trop Med Hyg* 2005; **73**: 199-203.
[92] Hopkins H, Kambale W, Kamya MR, Staedke SG, Dorsey G, Rosentha PJ. Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda. *Am J Trop Med Hyg* 2007; **76**: 1092-1097.
[93] Surabattula R, Vejandla MP, Mallepaddi PC, Faulstich K, Polavarapu R. Simple, rapid, inexpensive platform for the diagnosis of malaria by loop mediated isothermal amplification (LAMP). *Exp Parasitol* 2013; **134**: 333-340.
[94] Pakalapati D, Garg S, Middha S, Kochar A, Subudhi AK, Arunachalam BP, et al. Comparative evaluation of microscopy, OptiMAL(®) and 18S rRNA gene based multiplex PCR for detection of *Plasmodium falciparum* and *Plasmodium vivax* from field isolates of Bikaner, India. *Asian Pacific J Trop Med* 2013; **6**: 346-351.
[95] World Health Organization. *Monitoring antimalarial drug resistance*. Geneva: WHO; 2001.
[96] Shekalaghe S, Drakeley C, Gosling R, Ndaro A, van Meegeren M, Enevold A, et al. Primaquine clears submicroscopic *Plasmodium falciparum* gametocytes that persist after treatment with sulphadoxine-pyrimethamine and artesunate. *PLoS One* 2007; **2**: e1023.
[97] White NJ. Primaquine to prevent transmission of falciparum malaria. *Lancet Infect Dis* 2013; **13**: 175-181.
[98] World Health Organization. *WHO guideline for the treatment of malaria*. Geneva: WHO; 2006.
[99] Sharma YD. Molecular surveillance of drug-resistant malaria in India. *Curr Sci* 2012; **102**: 696-703.
[100] Lee JS, Lee WJ, Cho SH, Ree HI. Outbreak of vivax malaria in areas adjacent to the demilitarized zone, South Korea, 1998. *Am J Trop Med Hyg* 2002; **66**: 13-17.
[101] White NJ. Preventing antimalarial drug resistance through combinations. *Drug Resist Updat* 1998; **1**: 3-9.
[102] Dondorp AM, Fairhurst RM, Slutsker L, MacArthur JR, Breman JG, Guerin PJ, et al. The threat of artemisinin-resistant malaria. *N Engl J Med* 2011; **365**: 1073-1075.
[103] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2009; **361**: 455-467.
[104] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, et al. Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med* 2008; **359**: 2619-2620.
[105] World Health Organization. *Guidelines for the treatment of malaria*. 2nd ed. Geneva: WHO; 2010.
[106] World Health Organization. *Informal consultation on malaria elimination: setting up the WHO agenda, Tunis, 25-26 February 2006*. Geneva: WHO; 2006.
[107] Padmanaban G, Nagaraj VA, Rangarajan PN. Artemisinin-based combination with curcumin adds a new dimension to malaria therapy. *Curr Sci* 2012; **102**: 704-711.
[108] Nosten F, van Vugt M, Price R, Luxemburger C, Thway KL, Brockman A, et al. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet* 2000; **356**: 297-302.
[109] Barnes KI, Durrheim DN, Little F, Jackson A, Mehta U, Allen E, et al. Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. *PLoS Med* 2005; doi: 10.1371/journal.pmed.0020330.
[110] Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo AP, et al. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. *Lancet Infect Dis* 2010; **10**: 673-681.
[111] Zongo I, Dorsey G, Rouamba N, Tinto H, Dokomajilar C, Guiguemde RT, et al. Artemether-lumefantrine versus amodiaquine plus sulfadoxine-pyrimethamine for uncomplicated

falciparum malaria in Burkina Faso: a randomised non-inferiority trial. *Lancet* 2007; **369**: 491-498.

[112]Koram KA, Abuaku B, Duah N, Quashie N. Comparative efficacy of antimalarial drugs including ACTs in the treatment of uncomplicated malaria among children under 5 years in Ghana. *Acta Trop* 2005; **95**; 194-203.

[113]Ogungbamigbe TO, Ojurongbe O, Ogunro PS, Okanlawon BM, Kolawole SO. Chloroquine resistant *Plasmodium falciparum* malaria in Osogbo Nigeria: efficacy of amodiaquine+sulfadoxine-pyrimethamine and chloroquine + chlorpheniramine for treatment. *Mem Inst Oswaldo Cruz* 2008; **103**: 79-84.

[114]Sowunmi A. A randomized comparison of chloroquine, amodiaquine and their combination with pyrimethamine-sulfadoxine in the treatment of acute, uncomplicated, *Plasmodium falciparum* malaria in children. *Ann Trop Med Parasitol* 2002; **96**: 227-238.

[115]Basco LK, Same-Ekobo A, Ngane VF, Ndounga M, Metoh T, Ringwald P, et al. Therapeutic efficacy of sulfadoxine-pyrimethamine, amodiaquine and the sulfadoxine-pyrimethamine-amodiaquine combination against uncomplicated *Plasmodium falciparum* malaria in young children in Cameroon. *Bull World Health Organ* 2002; **80**; 538-545.

[116]O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, et al. Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. *Lancet* 2008; **372**: 1555-1562.

[117]Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJ, et al. Changes in malaria indices between 1999 and 2007 in the Gambia: a retrospective analysis. *Lancet* 2008; **372**: 1545-1554.

[118]Obonyo CO, Juma EA. Clindamycin plus quinine for treating uncomplicated falciparum malaria: a systematic review and meta-analysis. *Malar J* 2012; **11**: 2.

[119]Sacarlal J, Aide P, Aponte JJ, Renom M, Leach A, Mandomando I, et al. Long-term safety and effi cacy of the RTS,S/AS02A malaria vaccine in Mozambican children. *J Infect Dis* 2009; **200**: 329-336.

[120]Lell B, Agnandji S, von Glasenapp I, Haertle S, Oyakhiromen S, Issifou S, et al. A randomized trial assessing the safety and immunogenicity of AS01 and AS02 adjuvanted RTS,S malaria vaccine candidates in children in Gabon. *PLoS One* 2009; doi: 10.1371/journal.pone.0007611.

[121]Abdulla S, Oberholzer R, Juma O, Kubhoja S, Machera F, Membi C, et al. Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants. *N Engl J Med* 2008; **359**: 2599-2601.

[122]Garçon N, Heppner DG, Cohen J. Development of RTS,S/AS02: a purified subunit-based malaria vaccine candidate formulated with a novel adjuvant. *Expert Rev Vaccines* 2003; **2**: 231-238.

[123]Asante KP, Abdulla S, Agnandji S, Lyimo J, Vekemans J, Soulanoudjingar S, et al. Safety and efficacy of the RTS,S/AS01E candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial. *Lancet Infect Dis* 2011; **11**: 741-749.

[124]The RTS, S Clinical Trials Partnership. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med* 2011; **365**: 1863-1875.

[125]White NJ. A vaccine for malaria. *N Engl J Med* 2011; doi: 10.1056/nejme1111777.

[126]Olotu A, Lusingu J, Leach A, Lievens M, Vekemans J, Msham S, et al. Efficacy of RTS,S/AS01E malaria vaccine and exploratory analysis on anti-circumsporozoite antibody titres and protection in children aged 5-17 months in Kenya and Tanzania: a randomised controlled trial. *Lancet Infect Dis* 2011; **11**: 102-109.

[127]Epstein JE, Tewari K, Lyke KE, Sim BK, Billingsley PF, Laurens MB, et al. Live attenuated malaria vaccine designed to protect through hepatic $CD8^+$ T cell immunity. *Science* 2011; **334**: 475-480.

[128]O'Meara WP, Mangeni JN, Steketee R, Greenwood B. Changes in the burden of malaria in sub-Saharan Africa. *Lancet Infect Dis* 2010; **10**: 545-555.

[129]Na-Bangchang K, Karbwang J. Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. *Fundam Clin Pharmacol* 2009; **23**: 387-409.

[130]Trape JF, Tall A, Diagne N, Ndiath O, Ly AB, Faye J, et al. Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. *Lancet Infect Dis* 2011; **11**: 925-932.

[131]Roberts L, Enserink M. Malaria. Did they really say...eradication? *Science* 2007; **318**: 1544-1545.

[132]Nayyar GML, Breman JG, Newton PN, Herrington J. Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa. *Lancet Infect Dis* 2012; **12**: 488-496.

[133]Kappe SHI, Mikolajczak SA. Another shot at a malaria vaccine. *Science* 2011; **334**: 460-461.

[134]Douglas NM, Anstey NM, Angus BJ, Nosten F, Price RN. Artemisinin combination therapy for vivax malaria. *Lancet Infect Dis* 2010; **10**: 405-416.

[135]Maude RJ, Pontavornpinyo W, Saralamba S, Aguas R, Yeung S, Dondorp AM, et al. The last man standing is the most resistant: eliminating artemisinin-resistant malaria in Cambodia. *Malar J* 2009; **8**: 31.

[136]Cairns M, Roca-Feltrer A, Garske T, Wilson AL, Diallo D, Milligan PJ, et al. Estimating the potential public health impact of seasonal malaria chemoprevention in African children. *Nat Commun* 2012; **3**: 881.

[137]Alli LA, Adesokan AA, Salawu OA, Akanji MA, Tijani AY. Anti-plasmodial activity of aqueous root extract of *Acacia nilotica*. *African J Biochem Res* 2011; **5**: 214-219.

[138]Ajaiyeoba EO, Abiodun OO, Falade MO, Ogbole NO, Ashidi JS, Happi CT, et al. *In vitro* cytotoxicity studies of 20 plants used in Nigerian antimalarial ethnomedicine. *Phytomedicine* 2006; **13**: 295-298.

[139]Mandal S. Exploration of larvicidal and adult emergence inhibition activities of *Ricinus communis* seed extract against three potential mosquito vectors in Kolkata, India. *Asian Pac J Trop Med* 2010; **3**: 605-609.

[140]Schwartz L, Brown GV, Genton B, Moorthy VS. A review of malaria vaccine clinical projects based on the WHO rainbow table. *Malar J* 2012; **11**: 11.

[141]Arnott A, Barry AE, Reeder JC. Understanding the population genetics of *Plasmodium vivax* is essential for malaria control and elimination. *Malar J* 2012; **11**: 14.

[142]Feachem RGA, Phillips AA, Hwang J, Cotter C, Wielgosz B, Greenwood BM, et al. Shrinking the malaria map: progress and prospects. *Lancet* 2010; **376**: 1566-1578.

[143]Leslie T, Briceño M, Mayan I, Mohammed N, Klinkenberg E, Sibley CH, et al. The impact of phenotypic and genotypic G6PD deficiency on risk of *Plasmodium vivax* infection: a case-control study amongst Afghan Refugees in Pakistan. *PLoS Med* 2010; doi:10.1371/journal.pmed.1000283.