

## Review Article

# Old and Recent Advances in Life Cycle, Pathogenesis, Diagnosis, Prevention, and Treatment of Malaria Including Perspectives in Ethiopia

Dejen Nureye <sup>1</sup> and Solomon Assefa <sup>2</sup>

<sup>1</sup>Department of Pharmacy, College of Medicine and Health Sciences, Mizan-Tepi University, Mizan Campus, P.O. Box 260, Southwest, Mizan-Aman, Ethiopia

<sup>2</sup>Department of Pharmacology and Clinical Pharmacy, College of Health Sciences, School of Pharmacy, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Correspondence should be addressed to Dejen Nureye; [dejenureye@gmail.com](mailto:dejenureye@gmail.com)

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Malaria, caused by apicomplexan parasite, is an old disease and continues to be a major public health threat in many countries. This article aims to present different aspects of malaria including causes, pathogenesis, prevention, and treatment in an articulate and comprehensive manner. Six *Plasmodium* species are recognized as the etiology of human malaria, of which *Plasmodium falciparum* is popular in East and Southern Africa. Malaria is transmitted mainly through *Anopheles gambiae* and *Anopheles funestus*, the two most effective malaria vectors in the world. Half of the world's population is at risk for malaria infection. Globally, the morbidity and mortality rates of malaria have become decreased even though few reports in Ethiopia showed high prevalence of malaria. The malaria parasite has a complex life cycle that takes place both inside the mosquito and human beings. Generally, diagnosis of malaria is classified into clinical and parasitological diagnoses. Lack of clear understanding on the overall biology of *Plasmodium* has created a challenge in an effort to develop new drugs, vaccines, and preventive methods against malaria. However, three types of vaccines and a lot of novel compounds are under preclinical and clinical studies that are triggered by the occurrence of resistance among commonly used drugs and insecticides. Antiadhesion adjunctive therapies are also under investigation in the laboratory. In addition to previously known targets for diagnostic tool, vaccine and drug discovery scientists from all corner of the world are in search of new targets and chemical entities.

## 1. Introduction

The term *malaria* was derived from the Italian word “*malaria*” meaning foul air [1]. It is a protozoal blood infection caused by a mosquito-borne apicomplexan parasite, which is transmitted to humans during the bite of an infected female *Anopheles* mosquito species [2, 3]. The United States National Institute of Allergy and Infectious Diseases (NIAID) defines malaria as a disease caused by a parasite that lives part of its life in humans and part in mosquitoes [4]. This review aims to present all aspects of malaria in a coherent and comprehensive manner. An attempt was made to give introductory concepts regarding history, causative agents,

prevalence, and incidence of malaria. It also provides old and new notions about the cell biology, pathophysiology, diagnosis, and management of malaria in one umbrella including some tips from Ethiopia. In advance, we seek to summarize recent developments in drug, vaccine, and control measures of malaria.

Malaria is an ancient disease that could be traced back to the very earliest human history. It was accepted as a disease by Hippocrates in the fourth century BC [5]. In the early seventeenth Century, the Peruvian bark of *Cinchona* tree was known to treat fever [6]. In 1847, Heinrich Meckel identified black-brown pigment granules in the blood and spleen of an insane person [7]. Othmer Zeidler synthesized

Dichloro-Diphenyl-Trichloroethane (DDT) in 1874 for his thesis. Alphonse Laveran noticed parasites, which he called *Oscillaria malariae*, in the blood of a malaria patient in 1880 [8]. The genus plasmodium was portrayed by Ettore Marchiafava and Angelo Celli in 1885 [9]. The whole transmission cycle of the parasite was elucidated in 1897 by Ronald Ross. In 1898, Camillo Golgi and others demonstrated that human malaria was transmitted by *Anopheles* mosquitoes [10]. Chloroquine was discovered in 1934 by Hans Andersag. He called his compound resochin. In early 1950s, malaria was thought to be eliminated from the USA. Then after, human infection with *Plasmodium knowlesi* was recognized in 1965. Artemisinin was isolated from the plant *Artemisia annua* in 1971 [6, 10]. Next, a polymerase chain reaction- (PCR-) based malaria detection was depicted in the early 1990s, and meanwhile malaria rapid diagnostic tests (RDTs) were developed [11].

The genus *Plasmodium* (the causative agent for malaria) is thought to have originated from Dinoflagellates (photosynthetic protozoa). From more than 200 different species of Plasmodium, at least 13 species are pathogenic to humans [9]. Five of them, *falciparum*, *vivax*, *ovale* (two species), and *malariae*, are well-known etiology of human malaria. Moreover, disease with *knowlesi* occur in people when an *Anopheles* mosquito infected by a monkey bites humans [12].

Of these species, *falciparum* (dominant in East and Southern Africa) is mainly prevalent on the African continent and is responsible for most deaths from malaria. *Plasmodium vivax* has a wider geographic distribution. Although it can occur throughout Africa, the risk of infection with *vivax* is quite low there because of the absence of Duffy gene in many African populations [13]. There is, however, a growing evidence that *vivax* is being transmitted among Duffy blood group-negative inhabitants in Africa including Ethiopia [14].

Malaria is transmitted majorly through bites of the genus *Anopheles* mosquitoes, which includes more than 537 recognized species [15]. The two most efficient malaria vectors in the world, *A. gambiae* and *A. funestus*, are primary malaria vectors in Africa [16]. *A. gambiae*, *A. funestus*, and *A. pharoensis* were confirmed as the principal vectors in Ethiopia [14]. Due to residence of the parasite in RBCs, malaria can also be transmitted through blood transfusion, organ transplant, or shared use of needles or syringes contaminated with blood. A new-born baby may acquire congenital malaria before or during delivery [17]. Furthermore, malaria transmission is largely affected by global weather patterns, including *El Nino* and *La Nina* [18].

Around 44% of the world's population is at risk from malaria [19]. According to the latest estimates, 219,000,000 malaria cases, most (92%) from World Health Organization (WHO) African Region, occurred globally in 2017, and the disease led to 435,000 deaths, most (93%) of which were also in WHO African Region. Almost all (99.7%) cases due to malaria are resulted from *falciparum*. In 2017, the number of global malaria mortality in children less than five years is estimated to be 266,000 [20]. Pregnant women have increased susceptibility to *falciparum* malaria. In malaria-

endemic areas, *falciparum* contributes to 8–14% of low birth weight, which in turn decreases the chance of a baby's survival [6].

Global malaria case incidence was reduced by 59% in 2017. A reduction in mortality rates (44.1%) was also reported in this year [20]. Not only health related impact but also there is a severe economic burden in terms of lost days of work due to the disease. Of course, malaria is considered to take off 1.3% from the economic growth and 40% of public health expenditure of some African countries. It affects developing countries in many aspects including determent of tourism [21].

Malaria was one of the top ten as well as major infectious diseases in Ethiopia [22, 23]. About 28,548,422 people of Ethiopia live in the high risk area for malaria infection. In 2017, Ethiopian Federal Ministry of Health (FMOH) reported 1,530,739 confirmed malaria cases and 356 deaths [20]. Despite decreased malaria occurrence rate and death rate in Ethiopia since 2010 [20], high prevalence was observed in some areas in contrast to high household coverage of control interventions [24, 25]. This increment may be associated with individuals having poor socioeconomic status [26]. Ethiopia has achieved only half of the millennium development reduction target of malaria. For this reason, the country must strengthen its malaria control and treatment approaches to attain the sustainable development goals [27].

## 2. Life Cycle of Malaria Parasite

The human malaria parasite has a complex life cycle as shown in Figure 1. The motile infectious form, Plasmodium sporozoite, is passed to individuals when the insect bites the skin, probes for a blood vessel from which to feed, releases various vasodilators to increase its chance of finding a vessel and salivate into the blood to prevent clotting. Within 30–60 min of inoculation, the thread-like sporozoites are carried to the liver by the circulatory system [4, 5].

Over a period of 7–12 days, the sporozoites grow into schizonts and can develop up to 30,000 merozoites, which rupture the hepatocytes [28, 29]. On the other hand, some *vivax* and *ovale* sporozoites turn into hypnozoites, a form that can remain latent in the liver for months or years and cause relapses in infected people [30]. Interestingly, recurrence of *falciparum* malaria was reported in patients some years after leaving an endemic area. It tells that, at least occasionally, *falciparum* has a dormant stage [31–33]. Then, the asexual cycle begins (Figure 1), with the merozoites invading RBC to grow by consuming hemoglobin. Within the host RBC, the parasite undergoes development from the early ring stage to late trophozoite and then after mitotic divisions to the schizont stage, which contains 6 to 32 merozoites, depending on the parasite species [34].

When the erythrocytic schizont ruptures, the released merozoites continue the life cycle by invading other RBCs. Cyclical fevers are typically happening shortly before or at the time of RBC lysis as schizonts rupture to release new infectious merozoites. This occurs every 48 h in tertian malaria and every 72 h in quartan malaria infection. During

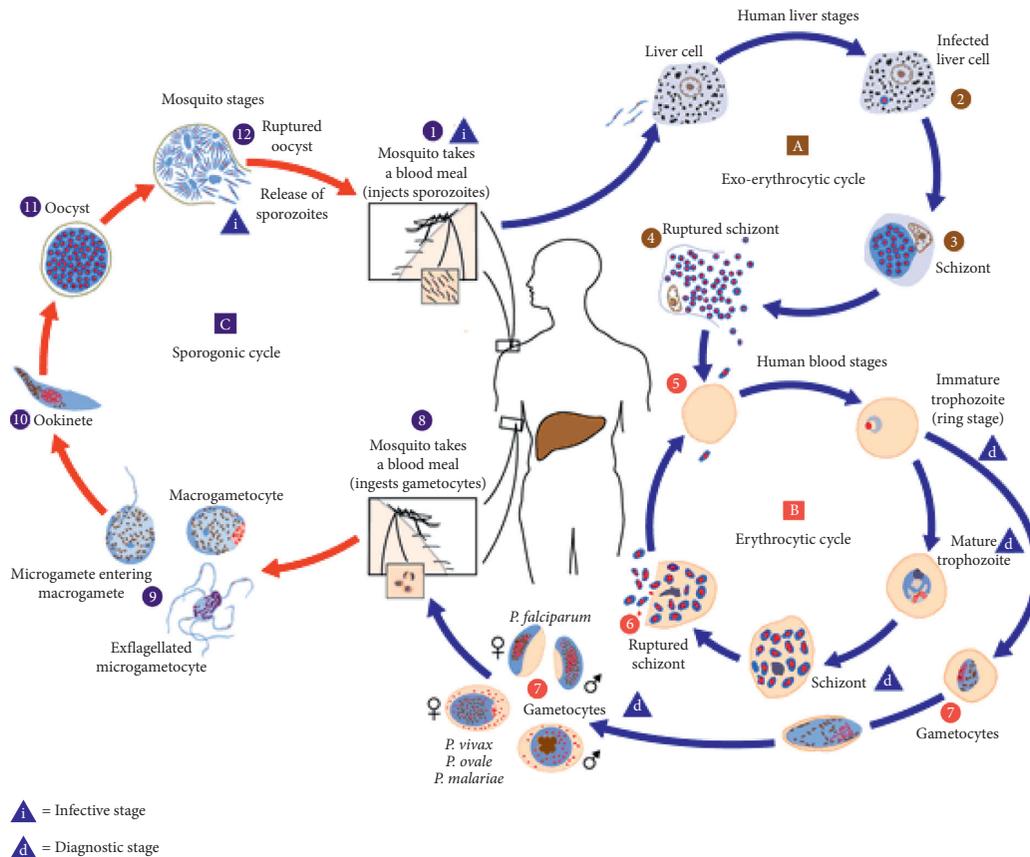


FIGURE 1: Life cycle of malaria parasites (adopted from [35]).

this repeated cycle, some merozoites differentiate into male and female sexual forms known as erythrocytic gametocytes with one nucleus and then awaiting the arrival of a blood-seeking female *Anopheles* mosquito [4, 34].

Then intake of gametocytes by the mosquito induces gametogenesis. The flagellated forms of microgametes, formed by exflagellation, penetrate or fertilize the macrogametes generating zygotes. The zygotes change into ookinetes and then become a round oocyst. Inside the oocyst, the nucleus divides repeatedly, with the formation of a large number of sporozoites and enlargement of the oocyst [28]. When the sporozoites are fully formed, the oocyst bursts, releasing the sporozoites into the haemocoel (the mosquito's body cavity). The sporozoites migrate to the salivary glands, thus completing the life cycle (Figure 1). Entrance of the sporozoites from the mosquito's salivary glands into a new human host perpetuates the malaria life cycle [6, 28].

### 3. Cell Biology and Pathogenesis of Malaria

The cell biology of *Plasmodium* is suggested to be similar to other eukaryotes since members of this genus are eukaryotic microbes. However, the whole biology of the parasite and its pathogenesis is not clearly known at the molecular and cellular levels [36, 37]. Here, we compiled old and recent facts to inform and click experts to involve them in developing vaccines, novel drugs, and malaria control tools, since somehow in-depth understanding on the biology of

malaria parasite is critical to engage into these activities. All Apicomplexa including malaria parasites are characterized by a set of apical organelles called rhoptries, dense granules, and micronemes. In *Plasmodium* spp., there are three invasive forms: sporozoite, merozoite, and ookinete involving the apical organelles localized at one end of the parasite [38].

First, the sporozoite injected into the host skin enters the blood stream and quickly accesses the liver by a process called traversal. Proteins necessary for traversal are reviewed in [39]. The coreceptors on sporozoites (e.g., thrombospondin (TSP) domains on the circumsporozoite protein (CSP)) that mediate invasion [40] bind specifically to glycoaminoglycan chains of the heparan sulfate proteoglycans (HSP) on hepatocytes and Kupffer cells [41]. At least, two receptors, CD81 and CD68, are found to be responsible for *falciparum* entry and invasion in hepatocytes. After penetrating space of Disse in the liver, sporozoites migrate through several hepatocytes and engage in a final invasion, with the formation of a parasitophorous vacuolar membrane (PVM). PVM is then ruptured by plasmodial proteolytic enzymes and merozoite egress from the infected hepatocyte to access blood circulation [42, 43].

Within the circulation, merozoite surface protein-1 (MSP-1) associated with the parasite membrane through a glycosylphosphatidylinositol (GPI) anchor does binds to the RBC surface proteins [44]. Eight other merozoite surface-bound GPI-anchored proteins interacting with RBC have been reviewed elsewhere [45]. Recently, CD55 (a protein on

the surface of RBCs) was identified that serves as an essential gateway point for malaria parasite into RBCs. This discovery opens up a new path for the development of therapies to treat and prevent malaria [46]. After binding to RBC, the merozoite reorients itself using apical membrane antigen 1 (AMA-1) so that the apical end of the parasite will locate adjacent to the RBC membrane with a transient RBC deformation. The contents of apical organelles are going to be expelled as the parasite invades [47, 48].

Following reorientation and microneme discharge, a junction was formed between the parasite and host cell using microneme proteins that recognize and bind to receptors in the host [37, 49]. Proteins in the neck of rhoptry such as RON2 are inserted into the host membrane and bind to AMA-1 to form the tight junction [50]. Then, the contact area becomes free of RBC membrane proteins. After this, a merozoite enzyme (serine protease) results in a localized disruption of the submembrane cytoskeleton and lipid architecture of RBC [51]. Formation of the junction triggers the release of rhoptry bulb, providing proteins and lipids required for the parasitophorous vacuole [52]. So, an incipient PVM will be formed in the junction area [53]. The junction between the parasite and host becomes like a ring and the parasite appears to move via this annulus as it enters the expanding parasitophorous vacuole [54]. Following doorway, the PVM and host cell membrane become closed [55].

The invasive forms of apicomplexan parasites are motile forms that crawl along the substratum by “gliding motility” (Figure 2). During invasion, the parasite literally crawls into the host cell via the moving junction. In gliding motility, the micronemes must be continuously released as the organism is moving and continuous formations of new junctions occur between the zoite and substratum. A myosin unique to the Apicomplexa would be anchored into the inner membrane complex (IMC) lying under the plasma membrane. The IMC-associated myosin will be interacted with actin as part of the glideosome. The various adhesins making up the moving junction (MJ) complex are then linked to the glideosome [55].

The myosin propels the actin filaments toward the posterior of the zoite. As myosin is anchored into the IMC, it does not move. Therefore, the transmembrane adhesins are pulled via the fluid lipid bilayer of the plasma membrane. Thus, the complex of adhesins and actin filaments is transported towards the posterior of the cell to produce forward motion of the parasite. When the adhesins reach the posterior end of the parasite, they are proteolytically cleaved and shed from the zoite surface and a trail of adhesive molecules is left behind the moving zoite on the substratum [37, 56].

Once inside the RBC, the parasite modifies the host cell to make it a more suitable environment. Formation of knobs, cytoadherence, and rosetting (Figure 3) are the major host cell changes which happen in the pathogenesis of malaria. Knob-associated histidine-rich protein (KAHRP) and erythrocyte membrane protein-2 (PfEMP2) are two of the several proteins which reorganize the host RBC submembrane cytoskeleton and induce knob formation [57]. A

polymorphic protein, PfEMP1, has been anchored to the knobs by KAHRP and has become exposed on the host RBC surface and functions as a ligand. Other cytoadherence ligands [58] are shown in the right side of Figure 3.

The red cells infected with mature forms of the parasites adhere to the capillary and postcapillary venular endothelium in the deep microvasculature which leads to sequestration of the parasites in various organs such as the heart, lung, brain, liver, kidney, intestines, adipose tissue, and placenta. This feature of the disease has been related exclusively to *falciparum* [59, 60]. However, it has also been seen in reticulocytes infected with *vivax* [61]. To adhere to the endothelium, PfEMP1 appears on the surface of the infected red blood cells (IRBCs) about 16 h after the invasion [62]. This antigenic variant molecule can bind to several adhesion receptors, as shown in the left side of Figure 3, expressed on the endothelial cells. Among these receptors, intercellular adhesion molecule-1 (ICAM-1) is a major sequestration receptor and serves as a rolling receptor [63, 64].

Sequestration is also seen during pregnancy when IRBC binds to placental chondroitin sulfate A (CSA), which is mediated by Variant Two chondroitin Sulphate A Antigen (VAR2CSA) [65]. So, placental malaria can cause miscarriage, intrauterine growth retardation, low birth weight, and congenital malaria [66]. Parasites sequestration provides them the microaerophilic venous environment that is better suited for their maturation, and adhesion allows them to escape clearance by the spleen and to hide from the immune system. IRBCs also adhere to uninfected RBCs to form red cell rosetting and to other parasitized RBCs to form agglutination [67].

In rosette formation, PfEMP1 has been shown to bind to complement receptor-1 (CR-1), heparin sulfate (HS), and ABO blood group [68, 69]. The lectin-like duffy-binding domain (DBL) of PfEMP1 can make strong adhesion with carbohydrate structures, particularly blood group A antigen [70]. That is why non O-blood groups are risk factors for life-threatening malaria through enhanced rosette formation [69, 71]. *Falciparum*, *vivax*, and *ovale* are all able to form rosettes [72, 73], but only those caused by *falciparum* have been associated with severe malaria [74]. *Vivax* and *ovale* show a marked penchant for young RBCs, while malariae prefers old cells. As a result, these parasites have low parasitemia level in the bloodstream. *Plasmodium falciparum*, however, can invade RBCs of all ages and produces very high parasitemia level [46, 75].

If the above-mentioned pathophysiologic process goes on uninhibited, it ultimately blocks blood flow, limits local oxygen supply, impedes mitochondrial ATP synthesis, and stimulates cytokine production, all these factors contributing to the development of a severe disease [76]. Additionally, the host RBC ruptures or lyses to enable parasite egress as subtilisin-like protease 1- (SUB1-) processed MSP-1 interacts with the spectrin network of the RBC cytoskeleton [77]. Along with lysis of IRBC, toxins (red cell membrane products, hemozoin pigment, and GPI) are also released into the blood and activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators. The systemic

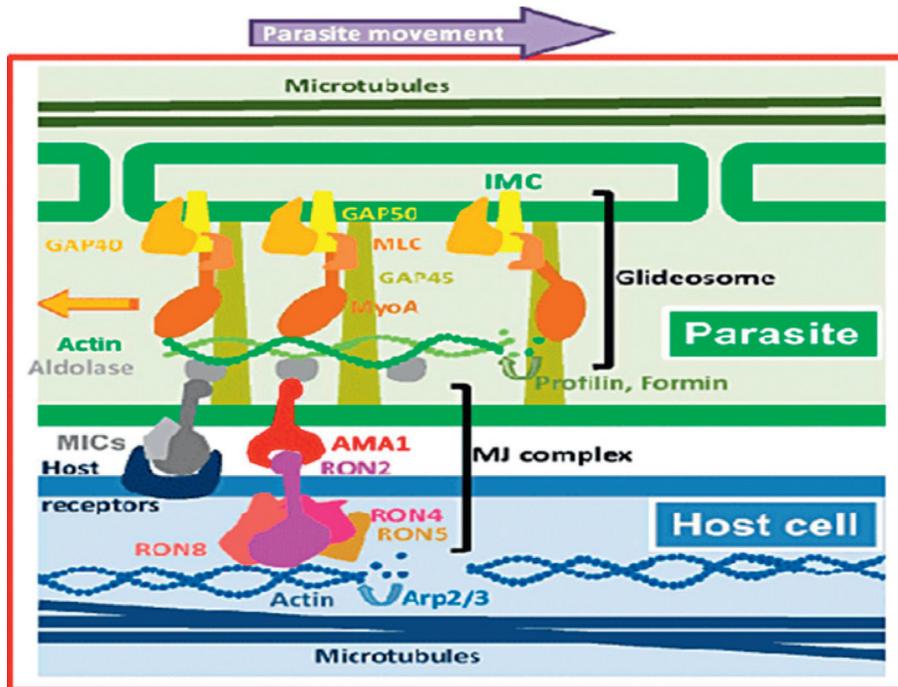


FIGURE 2: Model of the moving junction complex and glideosome driving gliding motility (GAP: gliding-associated protein, MyoA: myosin A, MLC: myosin light chain, and Arp2/3: actin nucleator complex) (adopted from [55]).

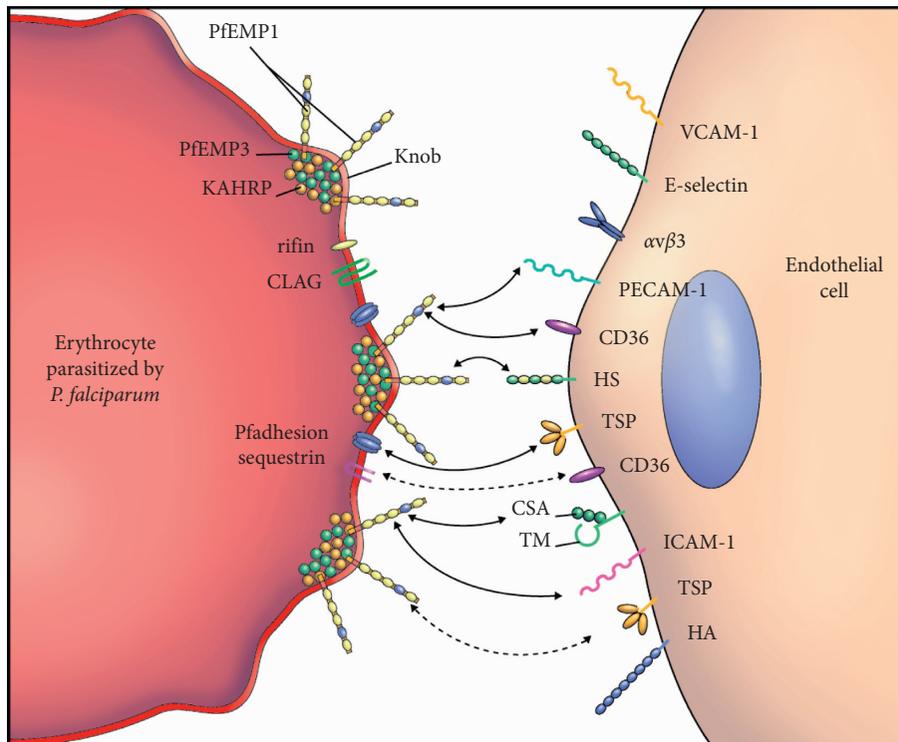


FIGURE 3: *Falciparum* cytoadherence (VCAM-1: vascular cell adhesion molecule-1,  $\alpha\text{v}\beta\text{3}$ : integrin, PECAM-1: platelet endothelial cell adhesion molecule-1, HA: hyaluronic acid, CLAG: clag9 ligand) (adopted from [7]).

manifestations of malaria including fever have been largely attributed to the released cytokines and toxins [78, 79].

In addition, the plasmodial DNA presented by hemozoin interacts intracellularly with the Toll-like receptor-9, leading

to the release of proinflammatory cytokines that in turn induce cyclooxygenase (COX-2) upregulating prostaglandins proceeding to induction of fever [80, 81]. Hemozoin has also been linked to induction of apoptosis in

developing erythroid cells in the bone marrow, thereby causing anemia [82, 83]. Likewise, Mawson hypothesized that the parasites emerge from the liver packed with vitamin A and use retinoic acid as a cell membrane destabilizer to invade the RBCs, causing hemolysis and anemia [84].

The clinical manifestations of severe malaria, caused especially by *falciparum*, are directly correlated with the induction of strong proinflammatory immune responses [85]. Hyperactive immune response is one of the major contributors to cerebral malaria vasculopathy, and fatal outcome is generally ascribed to sequestration of activated macrophages, parasitized erythrocytes, and platelets in cerebral vessels [86].

#### 4. Diagnosis of Malaria

Malaria must be diagnosed early and accurately to end up with an effective management of patients. Broadly, one can classify it into clinical and parasitological diagnoses. Clinical diagnosis is based on the patient's symptoms and on signs at physical examination [6, 13].

All of the suspected malaria should be confirmed with a parasitological diagnosis in all settings [13]. Light microscopy and RDTs are routinely employed methods for parasitological diagnosis of malaria. Detection of the parasites on giemsa-stained peripheral blood smears by light microscopy is used as the gold standard for diagnosis of malaria. As *knowlesi* and *malariae* have almost similar morphology, microscopy alone is insufficient to diagnose *knowlesi* [87, 88]. In case of *vivax*, *ovale*, and *malariae*, all development stages subsequent to the liver cycle can be seen in the peripheral blood. However, in *falciparum*, only ring forms and banana-like gametocytes are usually present in the peripheral blood since mature parasites become sequestered [89].

In areas where microscopy is not readily available, RDTs can be used and are based on the detection of antigens or enzymatic activities associated with the parasites. The most common antigens for RDTs are *P. falciparum* histidine-rich protein-2 (PfHRP2), specific for *falciparum* malaria, and two enzymes of the parasite glycolytic pathways, namely plasmodial lactate dehydrogenase (pLDH) and aldolase. LDH can be specific for *falciparum* or *vivax* malaria or it can be a variant pan specific (common to all six species). RDTs can also measure parasite antigens when mature parasites are sequestered. But, some isolates from the Amazon region, Africa, and India have been found lacking the PfHRP2, probably HRP2 gene deletion, which threatens the ability to diagnose and appropriately treat people infected with *falciparum* malaria [88, 89]. In 2005, single-species RDTs were introduced in Ethiopia. Years after, multispecies RDTs are being supplied by FMOH to health posts [90].

PCR-based methods, another parasitological diagnostic means, are the most sensitive test able to identify low levels of parasitemia, parasite species, or mixed infections, but not a suitable method for routine use. A species-specific loop-mediated isothermal amplification (LAMP) method has become widely accepted for identifying *knowlesi* infections. Besides, PCR is helpful as a research tool in epidemiological

studies, clinical trials, and for detection of molecular markers of drug resistance to antimalarial agents [11, 91].

The 4<sup>th</sup> parasitological method is the serology test based on detection of antibodies against malarial parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past exposure [6]. Newly developed rolling circle-enhanced enzyme activity detection (REEAD) and micromagnetic resonance relaxometric (MMR) test are amenable to deployment in field conditions and are highly accurate and cost- and time-effective [92].

Novel malaria diagnostic targets have been searched and include extremely conserved genes of proteins [93]. The most abundant heat-shock protein (HSP), HSP-70, has been investigated as a new diagnostic protein [94]. Another promising targets would be Plasmodium heme detoxification protein (HDP) for all species of Plasmodium, protozoan dihydrofolate reductase (DHFR) to detect *falciparum* and *vivax* spp., Glutamate-rich protein (GLURP), and high-mobility group box 1 (HMGB1) protein for *falciparum* diagnosis [95–97]. Recently, one study reported that infection with malaria makes children smell more inviting to *Anopheles gambiae* mosquitoes, which carry the disease. An increase in discharges of chemicals known as aldehydes accounted for much of the change in attractiveness. The finding might be valuable in the development of latest noninvasive diagnostic methods because it enables to diagnose carriers of malaria parasites using odors even if they do not feel sick to visit a health institution [98].

#### 5. Preventive and Control Measures of Malaria

**5.1. Vector Control.** Many countries are trying to get rid of malaria. In 2017, a total of forty-six countries reported fewer than 10,000 malaria cases [20]. Ethiopia planned to eliminate malaria by 2020 [99]. To do so, vector control is an effective measure that has to be taken. *Anopheles* mosquitoes can be reduced via the use of insecticide-treated bed nets (ITNs) and indoor spraying of residual insecticides (IRSs). Endophilic mosquitoes are readily controlled by IRSs. In contrast, exophagic/exophilic vectors are best controlled through source reduction (destruction of the breeding sites) and larviciding [16]. Those insecticides in use, mostly within African countries, are pyrethroids (recommended for use only on bed nets), organochlorines (e.g., DDT), organophosphates, and carbamates [100]. The effectiveness of insecticide-based vector control is endangered by malarial mosquitoes developing resistance for the insecticides used. However, long-lasting insecticidal nets (LLINs) remain effective despite resistance. The current WHO advice for resistance management in areas with LLINs is additive spraying, with nonpyrethroids used on a rotational basis [13].

Attractive toxic sugar bait (ATSB) methods are a new form of vector control measure that slays mosquitoes searching for essential sugar sources in the outdoor environment. These approaches use fruit or flower smell as an attractant, sugar solution as a feeding stimulant, and oral

toxin to destroy the mosquitoes [101, 102]. Spraying swarms with aerosols is another vector control method that caused an extraordinary reduction in mosquito density [103]. Individual bite protection methods (e.g., insect repellants and protective clothing) have also been used to reduce malaria transmission by mosquitoes [104, 105]. Jaleta et al. (2016) demonstrated that nonhost (chicken) volatiles can provide protection to mosquito-vectored diseases in combination with established control programmes [106].

Majority of malaria vector species display a more diverse behavior, feeding on livestock and humans. Mosquitoes nourishing on livestock could be targeted through treatment of livestock structures (e.g., IRS of cattle sheds) [107]. Direct treatment of cattle with insecticides by dipping, sponging, or spraying has also been shown to kill mosquitoes and to reduce malaria in the human population [108, 109]. Use of systemic veterinary insecticides that affect the mosquitoes upon blood feeding is another alternative. Ivermectin has been successfully tested in cattle and demonstrated to both killing mosquitoes and shorten the lifespan of the survivors [110, 111].

Remarkable ways to interfere transmission include disrupting steroid hormone signaling in mosquitoes [112], use of transgenic mosquitoes [113, 114], using of paratransgenesis for delivering anti-*Plasmodium* effector molecules [115], and/or transinfection of mosquitoes with symbiotic bacteria and fungi [116]. The use of a transgenic procedure can improve the sterile insect technique (SIT) for *Anopheles* induced by radiation [117].

Uniquely, some genetic abnormalities (host polymorphism) in RBCs confer resistance to malaria. This include, among others, deficiency in pyruvate kinase, polymorphic glycoporphins, ovalocytosis, spherocytosis, elliptocytosis, sickle-cell traits, thalassaemia traits, and glucose-6-phosphate dehydrogenase (G6PD) deficiency [118–120]. The study of these protective polymorphisms can provide clues concerning naturally occurring systems of host defense, which could be used to develop new therapeutic drugs to combat malaria [119].

Drugs also play a major role in preventing malaria transmission. Intermittent Preventive Therapy (IPT) using sulfadoxine-pyrimethamine (SP) is another malaria control tool in pregnant women, infants, and preschool children where transmission is seasonal [66, 121]. Repeated Ivermectin Mass Drug Administration (MDA) could also help to control transmission [122]. Chemoprophylactic drugs such as chloroquine, mefloquine, and doxycycline or the combination of atovaquone and proguanil (Malarone®) are usually used to prevent infection in travelers as they move from no-malaria areas to places where malaria is common [123].

Cotrimoxazole is being administered to patients with human immunodeficiency virus (HIV) to guard against *Pneumocystis jirovecii* pneumonia and has been shown to reduce malaria infections and is a potential candidate for use as prevention in HIV-uninfected pregnant women or children [124]. Methylene blue is an old parasitocidal agent with blood stage activity and has added benefit due to effects against mature male and female *falciparum* gametocytes [125]. Despite the

dearth of new compounds in development for chemoprevention only, DSM-265 demonstrates its potential as a prophylactic drug for travelers [126]. Note that blood-stage parasites surrounded by PVM proliferate within RBCs, and following each cycle of intracellular development, first rupture the PVM using SUB1 and then the RBC membrane through serine repeat antigen protease-like protein (SERA6) to allow egress of the merozoites, which invade fresh RBCs. Compounds that inhibit these proteases would target consecutive, interdependent steps in the egress pathway and so could form a new class of drug intended to prevent parasite proliferation and disease [39, 127].

**5.2. Malaria Vaccine.** The emergence and spread of drug and insecticide resistance has been limiting the current malaria control measures, thus safe and effective vaccine is required to achieve the world malaria eradication programme objectives. The justification for a malaria vaccine development is the observation that people living in endemic areas develop clinical protective immunity despite the morphological changes and antigenic variations during the parasite life cycle allows them to escape the protective immune responses of the host [128]. So far, three types of vaccine candidates have been intensively investigated: pre-erythrocytic vaccines to prevent blood-stage infection; blood-stage vaccines to clear parasitaemia and prevent clinical disease; and transmission-blocking vaccines to prevent infection of mosquitoes and interrupt malaria transmission in populations [129].

Pre-erythrocytic vaccines target the sporozoites and/or hepatic stages of the parasite. Some vaccines of this group are RTS,S/AS01 in phase IV clinical trial, *falciparum* sporozoite vaccine (PfSPZ) in phase II trials [130], *vivax* malaria protein 1 (VMP001/AS01B) in phase I/IIa trial [131], cell-traversal protein for ookinetes and sporozoites (CelTOS) [FMP012/GLA-SE or AS01] under phase I/IIa clinical trial [132], and vaccine of chimpanzee adenovirus expressing CS (CSVAC) [133], genetically attenuated parasite (GAP) vaccines and chemoprophylaxis vaccination (CVac) in Phase I clinical trial [130]. Using *in vitro* studies, *falciparum* liver-stage antigens (PfLSA-1, 2 & 3) and *vivax* liver-stage antigens (PvLSAs) are recognized as a novel candidate vaccine targeting infected hepatocytes [134, 135].

Several asexual blood stage vaccines, most target merozoite antigens, are in clinical researches. Candidates for erythrocyte-stage vaccine are AMA1 [136], erythrocyte-binding antigen (EBA-175) [137], MSP-1 [138], MSP-1<sub>19</sub> [139], MSP-2 [140], MSP-3 [96], and serine repeat antigen 5 (SERA5) [141]. None has resulted in clear clinical protection, probably due to the highly polymorphic nature of the vaccine structures [142]. But, efforts to enhance the efficacy either with a novel adjuvants [143] using viral vector prime-boost strategies [144] or by combining AMA1 and MSP1 [145] have been increasing even though new non-polymorphic *falciparum* ligands, CX3CL1-binding proteins (CBP1 and CBP2), are now revealed by Hermand and his coworkers, which provides a new opportunity for innovative vaccination approaches [146]. With further research, new

antigens, *falciparum* reticulocyte-binding protein homolog 5 (PfPRH5) and rhoptry-associated leucine zipper-like protein 1 (RALP1) having a potential to become blood-stage vaccine candidates, are being discovered [147, 148]. PfPRH5 is actually in phase I clinical study [130]. Currently, *falciparum* merozoite protein MSP4 (naturally induces a strong antibody response in malaria endemic areas) has created an interest to be included into candidate vaccines [149]. Multigravidae who have acquired antibodies against VAR2CSA are indeed protected from pregnancy-associated malaria after one pregnancy. Based on this finding, vaccine candidates directed against VAR2CSA are under optimization [150].

Transmission-blocking vaccines (TBVs) target surface proteins expressed on gametocytes, zygotes, and ookinetes to prevent parasite development in the mosquito mid gut by specific host antibodies, complement proteins, and cytokines [151]. The vaccine candidates in this group include the gametocyte antigens (Pfs48/45 and Pfs230) [152], *falciparum* ookinete surface antigens (Pfs25 and Pfs28) [153], and their *vivax* homologues Pvs25 and Pvs28 [154]. Other more recently known targets of curiosity include Pfs47 (implicated in parasite immune evasion in the mosquito vector) [155] and PfHAP2 which are expressed on the male gametocyte and microgamete [156]. Different vaccines with their formulation concerns, classified within the above-mentioned three vaccine categories and beyond the scope of this article, are discussed in detail elsewhere [132, 157].

## 6. Treatment of Malaria

**6.1. Traditional Medicine.** Traditional medicine (TM) use varies among countries depending on a number of factors. In Singapore and the Republic of Korea where the conventional health-care system is quite well established, 76% and 86% of the respective populations still commonly use TMs. About 90% of general hospitals provide TM services for both outpatients and inpatients in China. Over 100 million Europeans are TM users [158]. In developing countries, 80% of the people almost exclusively use TMs. Virtually, 80% of the population living in Ethiopia is dependent on traditional medicine which essentially involves the use of plants [159, 160].

More than 1,200 plants that possess antimalarial activities are reported worldwide [161]. For example, *Ampeloziziphus amazonicus* and *Strychnopsis thouarsii* were commonly used in malaria-endemic areas of Brazil and Madagascar, and their antiparasitic activities have been demonstrated. It is probable that some of antimalarial plants contain as yet undiscovered active constituents [162].

Ethiopia is rich in a wide range of tropical habitats, remarkable biodiversity, and use of traditional remedies for the treatment of various ailments [163]. Studies conducted on numerous traditionally claimed Ethiopian medicinal plants confirmed their antimalarial activities including *Phytolacca dodecandra* [164], *Justicia schimperiana* [165], *Artemisia abyssinica* [166], *Vernonia amygdalina* [167], *Buddleja Polystachya* [168], *Strychnos mitis* [169], *Echinops kebericho* [170], *Aloe trichosantha*, *Cadaba rotundifolia*

[171], *Adhatoda schimperiana*, *Piper capense* [172], and *Gardenia ternifolia* [173].

**6.2. Conventional Medicine.** Malaria can lead to fatal outcomes in only few days, thus treatment should be started as soon as possible. The main targets of current antimalarial drugs are asexual blood stages of the parasite, responsible for the malarial symptoms [89]. Nowadays, the available antimalarials can be grouped into five classes according to their chemical structure and biological activity: (i) quinoline-based antimalarials: 4-aminoquinolines (chloroquine, amodiaquine, and piperazine) and 8-aminoquinolines (premaquine and tafenoquine); (ii) arylaminoalcohols—quinine, mefloquine, halofantrine, and lumefantrine; (iii) antifolate compounds (pyrimethamine, proguanil, dapsone, and sulfadoxine); (iv) artemisinin and its derivatives: first generation (dihydroartemisinin, artesunate, arteether, and artemether) and second generation (artemisonone); and (v) hydroxynaphthoquinone-atovaquone [89, 174].

Chloroquine is a blood schizonticidal agent and the drug of choice for all malarial parasites except for chloroquine-resistant *Plasmodium* strains. Although almost all strains of malariae are susceptible, *falciparum*, *vivax*, and even some *ovale* strains have been reported as resistant to chloroquine [35]. Chloroquine resistance for *falciparum* is due to point mutations in the gene encoding chloroquine resistance transporter (PfCRT) protein, resulting in reduced drug accumulation in the food vacuole [175]. Drug resistance to chloroquine has been reported in Ethiopia [89]. Amodiaquine is effective against some parasite strains that are resistant to chloroquine, although some cross resistance exists [176]. Piperazine also has an excellent activity on chloroquine-resistant species [177].

Primaquine (tissue schizonticidal agent) is effective against the hypnozoites of *vivax* and *ovale* malaria and can kill gametocytes and consequently block the malaria transmission. Therefore, its effect on oocyst and sporozoite formation (and thus onward transmission of treated infection) precedes its effect on gametocytes carriage. It has weak activity against the asexual blood stage of *vivax* malaria. Primaquine is indeed used to achieve complete elimination of relapsing malaria due to *vivax* or *ovale*, in combination with a blood schizonticide for the erythrocytic parasites [12].

Quinine kills large ring and trophozoite asexual parasites and is gametocidal against *vivax*, *ovale*, and *malariae* but not *falciparum* malaria [178]. Mefloquine is also a blood schizonticide, active against the erythrocytic stages of all malaria parasites [179]. Proguanil is a biguanide compound that is active against all stages of *Plasmodium* [180]. SP has been the drug of choice for IPT in first and second trimester pregnancy and in infants living within malaria-endemic areas [181]. In combination with amodiaquine, SP is also used for seasonal malaria chemoprevention in children [12]. It is active predominantly against later development stages of asexual parasites. Resistance is caused by point mutations in parasite enzymes namely, dihydropteroate synthase (DHPS)

and DHFR [182]. Atovaquone is active against all *Plasmodium* species. It is ubiquinone analogue and acquires resistance related to a single mutation of cytochrome b gene of the parasite [183].

Artemisinin (endoperoxide sesquiterpene lactone) is a potent and fast acting blood schizonticidal killing all parasite stages. *Falciparum* resistance to them has now been detected in 5 countries in the Greater Mekong subregion: Cambodia, Lao People's Democratic Republic, Myanmar, Thailand, and Vietnam. These resistant strains have the capacity to spread to different parts of the world including Ethiopia and to subsequently become a global threat for malaria control and treatment [12, 184].

WHO recommends artemisinin-based combination therapies (ACT) for the treatment of uncomplicated malaria caused by *falciparum* parasite or by chloroquine-resistant *vivax*, *ovale*, *malariae*, and *knowlsi*. Atovaquone-proguanil may be considered for the treatment of uncomplicated malaria in travelers outside malaria-endemic areas. Quinine plus clindamycin is used for uncomplicated malaria treatment in the first trimester of pregnancy [12]. In Ethiopia, artemether-lumefantrine (Coartem™) is suggested as the first-line drug for uncomplicated *falciparum* malaria and chloroquine for other species, but oral quinine is considered as a second option [90].

More recently, injectable artesunate has become the treatment of choice for severe malaria worldwide in infants, children, lactating women, and pregnant women in all trimesters. After 24 h, the treatment should be completed with oral ACT [12]. Quinidine plus doxycycline, tetracycline, or clindamycin is the preferred drug in the USA to treat severe malaria [185]. Coming to Ethiopia, injectable artesunate is the preferred drug, and intramuscular artemether is an alternative drug. If these two drugs are not available, injectable quinine can be used to manage severe malaria [90].

**6.3. Drugs in the Pipeline.** The development of resistant strains and lack of new drugs are the limiting aspects in the fight against malaria. These factors trigger the continuing need for research of new classes of antimalarial agents and a re-examination of the existing antimalarial drugs. Hence, ozonides (synthetic peroxides) are proved to be useful substitutes for artemisinin. The first-generation ozonide OZ277 subsequently called arterolane was developed through a partnership between Medicines for Malaria Venture (MMV) and Ranbaxy. In 2012, Ranbaxy launched the combination of arterolane maleate and piperaquine phosphate as a 3-day treatment in India. After a limited Phase III programme, the combined drug has got approval under the trade name Synriam in India in 2013, followed by approval in seven African Nations in 2014 [129, 186].

Multiple novel combination therapies, including azithromycin-chloroquine [187], pediatric pyronaridine-artesunate, pediatric dihydroartemisinin in piperaquine [188], and trimethoprim-sulfamethoxazole [129] are in phase III trial. Tafenoquine (primaquine analog) is currently being tested in pivotal Phase III trial and has proven activity against hypnozoites [189]. It has the same G6PD deficiency liability, but

has the advantage of being a single-dose treatment [190] and possesses higher activity than primaquine [191].

Many new drugs are in phase 2 clinical development. The second-generation peroxide OZ439 (artefenomel) which has blood stage activity for *falciparum* and *vivax* malaria [190] is being tested in Phase IIb combination trial with piperaquine and is also tested with ferroquine (4-aminoquinoline), both in partnership between MMV and Sanofi [129]. The new organometallic drug ferroquine (SR97193) was found to be active against chloroquine-resistant strains and is currently undergoing Phase II clinical trials as combination therapy with artesunate [190]. Novartis currently has two new antimalarials, KAE609 (Cipargamin) and KAF156, in Phase II clinical testing. KAE609 (belongs to spiroindolone) has an inhibitory effect on *falciparum* cation channel (PfATPase4). It is blood schizonticide for *falciparum* and *vivax*. KAF156 (class of imidazolopiperazines) is with potential to treat and prevent malaria since it acts at multistage of the parasite life cycle [192, 193]. This drug is now joined phase IIb trial in combination with lumefantrine [194, 195].

DSM265 is another compound in phase II trial that inhibits *falciparum* dihydroorotate dehydrogenase (PfDHODH) and *vivax* dihydroorotate dehydrogenase (PvDHODH) enzymes [195]. This new chemical is a long acting with blood and liver stage activity [193]. Artemisone, a drug in Phase II study, provides a single-dose cure in Aotus monkeys infected with *falciparum* malaria at 10 mg/kg when combined with mefloquine 5 mg/kg [196]. Fosmidomycin, a natural antibacterial agent that inhibits an enzyme involved in the synthesis of isoprenoids (1-deoxy-D-xylulose 5-phosphate reductoisomerase) [161], is under combination therapy trial with piperaquine in phase II [188] in order to kill blood schizonts of uncomplicated *falciparum* malaria [191]. AQ-13, a modified chloroquine in phase II [186], retains activity against chloroquine-resistant parasites [197].

A phenothiazine derivative methylene blue is being developed (phase II) in combination with artesunate-amodiaquine as a strategy to protect against emergence of artemisinin resistance secondary to its *falciparum* schizonticidal effect and reduce transmission owing to gametocytocidal activity. Methylene blue acts by inhibiting *falciparum* glutathione reductase and as a result, prevents haem polymerization [126]. Polysaccharide heparin analogue Sevuparin (DF02) which is taken as an adjunctive therapy retains the antiadhesive effects of heparin without the antithrombin properties and has been shown to block merozoite invasion, cytoadherence, and rosetting [198]. MMV39048 is an aminopyridine currently in Phase 2a (NCT02880241) trial, and its target was identified to be lipid phosphatidylinositol 4-kinase (*Pf*PI4K). This drug has destructive activity on multiple stages of the parasite with possible efforts for chemoprevention [193, 199]. Albitiazolium (T3/SAR97276) has also reached Phase II clinical study. It acts mainly by deterring the transport of choline into the parasite [200].

An additional treatment panorama that has recently been entered into human clinical trial is quinoline-4-carboxamide DDD107498. It is an inhibitor of peptide elongation factor 2 with activity against pre-erythrocytic and

blood stages as well as mature gametocytes [201, 202]. A dihydroisoquinolone, SJ733, which inhibits gametocytogenesis as well as blood schizonts for *falciparum* and *vivax*, is now in human trial. It binds to a malaria parasite protein that serves as a sodium pump to interfere with the protein and sodium ions build up [203, 204]. Additionally, CDRI 97/78 (trioxane), ACT-451840 (phenylalanine-based compound), P218 (diaminopyridine and DHFR inhibitor), and GSK369796 (N-tert-butyl isoquine) are also among drugs under phase I study [129, 193].

Very recent analysis has predicted that lead compounds in a preclinical study have 8% possibility to become a registered product [205]. Most of them (e.g., SAR121, DM1157, and AN 13762) are blood schizonticidal except UCT 943 and NPC1161B, which has multistage activity [193]. Triaminopyrimidine MMV 253 that inhibits *Plasmodium* ATPase and aminomethylphenol JPC-3210, active against multidrug resistant *falciparum*, are long-acting agents in an early preclinical experiment [206, 207]. SC83288 (an amicarbalide derivative) is the only agent in preclinical investigation that are going to treat severe malaria [208]. It is also possible to list Genz-668764, ML238, ACT-213615, and TDR84420 within the new chemical entity group [209].

Furthermore, a pyrazoleamide 21A092 which targets sodium channel (ATPase4) like KAE609 and SJ557733 is in preclinical discovery phase [210]. Dantrolene was identified as a novel inhibitor of plasmodial surface anion channel (PSAC), and it may be a lead compound for antimalarial drug development [211]. Acridinones such as WR249685 and T3.5, a new class of selective malaria parasite mitochondrial *bc1* inhibitors, had a great potential to become novel antimalarial drugs [212, 213]. The study conducted by Wilson et al. (2015) identified for the first time that macrolide antibiotics inhibit *Plasmodium* species merozoite invasion into erythrocytes *in vitro*. This result directs the development of safe and effective macrolide antibiotics with dual modalities to combat malaria and reduce the parasite's options for resistance [214]. Antiadhesion adjunctive therapies including levanisole are under research in the laboratory [69]. *In vivo* and *in vitro* studies showed that an acriflavine (antibacterial and anticancer drug) impairs DNA replication foci formation in *Plasmodium berghei* and affects the enzymatic activities of apicoplast-specific Gyrase protein. This interesting work tells us the potential of this old drug to become a future antimalarial drug [215]. Now, a team of researchers has discovered thioredoxin enzymes which are different from the human one but critical for the survival of plasmodium by balancing redox state inside the parasite. So, the team is working with industry partners to create new drugs which will effectively target this enzyme and kill the parasite without affecting the human host [216].

## 7. Conclusion

Malaria, an ancient human disease, remains an important cause of illness and death in children as well as adults in endemic countries. *Falciparum* and *vivax* malaria produce a big challenge in the health of the community. Regardless of

decrement in prevalence and incidence of malaria, its transmission is still dynamic around the world. Therefore, malaria control requires an integrated approach including prevention primarily vector control and prompt treatment with effective antimalarial agents. However, an increasing resistance towards control measures and the currently available antimalarial drugs is a challenge to fight against malaria. Despite decades of intense research, no licensed malaria vaccines are available until now. Although many drugs are in the pipeline, most of them are not able to kill both gametocytes and hypnozoites. If the past instance is an indicator, resistance to the conventional antimalarials will spread to Africa including Ethiopia. Success and resistance are creating malaria scenery that requires new tools and approaches. Thus, the globe is in an urgent need of new, safe, and effective insecticides and drugs, as well as vaccines that can take over the currently resistance-prone phenomenon. In Ethiopia, a lot has to be performed to forward the malaria elimination plan.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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