Eradication of Malaria: Present Situations and New Strategies

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Author's contribution

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ABSTRACT

Malaria is a serious disease caused by the protozoon parasite Plasmodium and transmitted by the female Anopheles mosquito as a vector. P. falciparum is the gravest infection for all other species P. ovale, P. vivax, P. Malariae and P. knowlesi in terms of morbidity or mortality, which is why most research focused on P. falciparum. The disease affects about 300-500 million people, mostly in the tropics. In regions with a weak economic downturn in tropical and subtropical capital, morbidity and mortality have elevated. Malaria remains a persistent threat in recent research. At the beginning of the 20th century, scientists tried to describe a successful way of eradicating malaria. However, the presence of drug resistance and social and environmental problems, no acceptable and positive future solution has been pointed out. Several studies have highlighted the need to establish advanced nano-biotechnology treatment, novel anti-malarial drug targets, an efficient malaria vaccine technique, and Anopheles gene editing, which opened the door to a healthy, environmentally friendly malaria treatment method over the last two decades. In recent years, the use of mosquito microbiota has shown great potential for cutting down the transmission of mosquito-borne pathogens. This review aims to cover important issues in malarial eradication as rapid diagnostic technology, novel anti-malarial drug targets, Anopheles gene editing, use of mosquito microbiota, and recent vaccines.
Keywords: Plasmodium; diagnostic technology; novel anti-malarial drug targets; nano-biotechnology; Malaria eradication, Anopheles gene editing; recent vaccines.

1. INTRODUCTION

Epidemiology (prevalence, genomic epidemiology, life cycle and new strategies for eradication): Malaria is a parasitic infectious disease most prevalent in Sub-Saharan Africa, Asia, and South America. According to a study published by the World Health Organization (WHO) in 2018, there were 228 million malaria infections and 405,000 deaths globally in 2018, with an estimated 219 million cases in 2017 compared to 217 million in 2016 and 239 million in 2010. Annually nearly half a million deaths and infections in 228 million people were reported in 2019. The most significant burden of malaria morbidity is still in the African Region reported by the WHO [1]. Malaria will be eradicated by 2030, according to the World Health Organization [2].

Malaria affects over half of the world’s population, with most cases (93%) and fatalities (94%) occurring in Africa. Malaria is a key contributor to poverty and a substantial impediment to economic growth, particularly in Africa [3].

*Plasmodium falciparum, Plasmodium malariae, Plasmodium knowlesi, Plasmodium ovale* (*P. ovale curtisi* and *P. ovale wallikeri*), *Plasmodium cynomolgi*, and *Plasmodium vivax* are the six species of this genus that cause human infection. *P. falciparum* is the most deadly and widespread *Plasmodium* species, accounting for 99.7% of infections in Sub-Saharan Africa and killing non-immune youngsters at an unprecedented rate. In the Americas, *P. vivax* is the most prevalent, accounting for 75 percent of cases [1,4]. *P. vivax* was found to be responsible for 3.4 percent of all estimated cases worldwide [5,6].

The number of *P. falciparum* and *P. vivax* infections in Asia and Oceania is almost equal. Although their prevalence is modest, *P. ovale* and *P. malariae* are widespread [7]. *P. knowlesi*, a simian malaria parasite found mostly in long-tailed and pig-tailed macaques is another species. In Southeast Asia, however, zoonotic human illnesses have been documented [8].

*Plasmodium* species switches between vectors and hosts and need various zoite shapes for invading specific types of cells in particular phases (Fig. 1) [5,9]. In humans, the parasites first grow and replicate in the liver cells and then in the red blood cells [10-11].

The cell transversal via sinusoidal barrier was suggested to be essential to the infection by preparing sporozoite for hepatocyte invasions, which are not necessary for hepatocyte invasion but rather for intra-hepatocyte growth. When hepatocyte infection has developed, it is transformed for 2– 10 days into an exoerythrocytic or liver stage (LS), resulting in the sporozoite release into the bloodstream of up to 40,000 hepatocytes vesicles known as merozoites. Once released, free merozoites invade erythrocytes quickly and dynamically. Merozoites secreted from the erythrocytic stage of infection enter red blood cells (RBCs) and expand from the rings to trophozoites and schizonts stages of development. Egressing schizonts release merozoites, which perpetuate the asexual cycle. As erythrocyte development takes place over the subsequent four-hour period, the formation of 16-32 merozoites results in cell division (schizogony), which leads to erythrocyte membrane destruction and parasite explosion. Several parasites undergo a developmental transition during schizogony in the bloodstream, which initiates sexual development into the formation of male and female gametocytes [12-13].

Female *Anopheles* mosquitoes consume the gametocytes during a blood meal. Then progresses via the midgut to the ookinete, which then transitions into the salivary glands as sporozoites, ready to be injected after a blood meal to start an infection in humans [14].

A micro and macrogamete fusion is used to build a zygote that transforms for 24 hours into an ookinete which finds its way through a mosquito midgut epithelium where asexual replication is occurring, and in the midgut, they become extracellular male and female gametes over a 15-day cycle [5]. Malaria transmission from humans to mosquitoes depends on sexual development. The injection of anticoagulant saliva with sporozoites into another person these sporozoites move to the liver begins a new process when the infected *Anopheles* mosquito takes a blood meal [9,15].
Malaria control necessitates a coordinated approach that includes early and quick diagnosis, vector control, effective and safe anti-malarial drugs, and effective vaccinations [17]. However, the elimination of the disease is difficult since current control methods do not protect against outdoor mosquitoes, and when people are active, repellents can provide a personal protection solution [18]. Eliminating or eradicating malaria also requires developing new anti-malarial medicines and more effective vaccines for malaria eradication [6,19].

**Diagnosis:** The efficacy of malaria therapy is dependent on early diagnosis and suggestions for the best treatment plan [20]. Early diagnosis and treatment of malaria are critical for preventing the disease from progressing to a severe form and, as a result, lowering death, especially among children under the age of five [5].

**Clinical diagnosis:** Malaria is a life-threatening emergency, and suspected malaria patients should be urgently assessed [21]. For clinical diagnosis, physicians' signs and symptoms and physical examination of patients play a key role [22].

Malaria symptoms include fever/sweats/chills, malaise, myalgia, headache, and diarrhea, however it may not affect all individuals [21,23]. Fever (38.5°C or more and history of fever within 48 hours) is a marked symptom caused by erythrocyte rupture and release of parasites every 2 to 3 days according to species of *Plasmodium*. The higher the parasitemia, the worse it is. A higher count of parasitemia was also linked to fever, vomiting, dizziness and weakness. Fever and vomiting have been described as the most common symptoms of malaria. In addition, in patients with the first episode of malaria, symptoms such as chills, cough, and sweat frequently appear [24].

Hemoglobinuria is a rare complication of severe malaria that could contribute to acute kidney injury (AKI). Severe *Plasmodium knowlesi* malaria may be manifested with jaundice, respiratory distress, metabolic acidosis, and AKI. Renal complications of malaria are primarily caused by hemodynamic dysfunction and by the immune response. Hepatomegaly, jaundice and hepatic dysfunction complications may also lead to acute renal injury [5-26]. Complications in clinics are less common than in laboratories. Thrombocytopenia is the most common complication in the laboratory. Severe thrombocytopenia is a major phenomenon in complicated malaria patients, regardless of parasite types [27].

**Laboratory diagnosis:** Ineffective early diagnostic detection tools have hindered different control techniques, particularly in low parasitemia and low-transmission surveillance. The capability to discover asymptomatic people is a major impact on the dynamics of transmission, control
and possibly elimination of malaria. In addition, diagnostic trials can aid providers in health to further examine other febrile disease etiology; prevent serious diseases and probable mortality; minimize presumed usage and side-effects of anti-malarial medicine; and guard against quick drug resistance emerging and spreading [28].

Diagnostic approaches are typically heterogeneous in malaria-endemic areas. Therefore, the diagnosis is mainly performed using traditional procedures (microscopy and antigen detection in recent years, with quick diagnostic assays) [24].

2. MICROSCOPY

Microscopy offers several advantages, including great sensitivity and the ability to identify parasitemia and species, but it takes a long time and requires the employment of a highly experienced microscopist. In addition, due to poor parasitemia sensitivity, cases are frequently misdiagnosed or undetected, leading to incorrect or delayed treatment. Microscopic inspection of Giemsa-stained blood films, on the other hand, remains a key means of diagnosis in malaria case management and serves as a research reference standard [24,29].

Automatic machine-learning image recognition using convolutional neural networks (A digital microscope prototype device) can overcome such disadvantages with an algorithm. Autoscope has being evaluated for its microscopy potential. Autoscopic diagnostic performance-matched routine microscopy when the slides had adequately designed blood volumes; however, their performance on slides with smaller blood volumes was less than routine microscopy [30].

Rapid Diagnostic Test (RDTs): It is important today for malaria diagnostic technicians to identify Plasmodium species in every patient with fever using either malaria microscopy with stained slides or malaria rapid diagnostic tests (RDTs) (thick blood films or blood smear) [24,29,31]. Antigen detection (e.g., immunochromatography or RDTs) and nucleic acid detection are two additional fast diagnostic techniques accessible [32].

The Lancet Committee of eradicating malaria by 2050 stressed the relevance of RDTs as key instruments to identify asymptomatic illnesses and illnesses in pregnant women [2]. This method uses an antibody on a lateral flow immunoassay technology incorporated onto a cassette to identify parasite-specific biomarkers in a single cost-effective, easy, and quick step (15-20 minutes) [33]. A drop of blood is added in a check hole, a few buffer drops are put in the second hole, and the results can be read 15 minutes later. The binary code for conducting the tests is provided with control lines that determine positive or negative outcomes, similar to pregnancy tests [34]. RDTs are simple to use in resource-constrained and difficult-to-reach situations. In comparison to microscopy and PCR, staff training is significantly less intense [35].

These characteristics have increased RDTs' popularity at point of care (POC) application significantly since their inception, with the global distribution reaching an estimated 1.92 billion RDT units between 2010 and 2017 [36-37]. Africa is the largest customer, accounting for more than 80% of overall RDT sales (223 million out of the 276 million units) in 2017 [38].

Even though RDTs have dominated point-of-care tests (POCTs) for malaria detection, their detection limit is comparable to that of microscopy with Giemsa staining, and their sensitivity and specificity for species other than P. falciparum are lower. Due to parasites’ poor production of pan-aldolase, RDTs with the enzyme have limited sensitivity. As a result, pan-aldolase and PfHRP-2 are only found in a few RDTs. Furthermore, the risk of false positives and/or negative findings is a drawback of RDTs [20,25,39,40]. The RDT approach does not allow for the measurement of parasitemia, and its sensitivity has diminished when the number of parasites per liter has dropped below a certain threshold, such as 100 parasites/L [32]. As a result, tracking treatment efficacy is difficult, identifying carriers is hard, and the performance of different RDT brands varies, potentially reducing the method’s reliability [41]. Because pHRP-2 may be detected in the blood for up to 30 days after effective treatment and eradication of an active infection, it may create false positive findings [35]. However, large scales might provide false negative results in low parasitemia situations [24,29].

Currently, a sensitive fast diagnostic test is being created, which might have a detection limit of up to 10 times that of current RDTs, allowing it to identify disease incidence up to 1/2 day earlier than current RDTs [42]. The use of parasitized Plasmodium
RBCs as a method of circumventing the lack of recognized malaria biomarkers was discussed. Even with low parasitemia, the parasitized RBC populations rise to around 10,000 cells/L with 0.2 percent parasitemia and 250,000 – 500,000 infected cells/mL with 5-10 percent parasitemia [43]. Based on this information, a new microfluidic SELEX (I-SELEX) was developed to discover a collection of aptamers that bind to various epitopes found on parasitized RBC surfaces in a discrete manner [44]. In another work, researchers used gold nanoparticles AuNP modified screen-printed electrodes to immobilize monoclonal antibodies as capture elements for malaria-infected cells [45]. Impedimetric changes were observed throughout a 102–108-cell/mL linear response range of infected RBCs due to monoclonal antibodies’ interaction with parasitized RBCs distinct from infected normal RBCs.

The Lancet committee also stressed the need for new diagnostic technologies that do not require a blood sample from a finger prick. This difficulty is solved by diagnostic tests based on immunoassays, which are simple to perform and interpret and do not require complicated equipment or expert support. They are also quick (less than 10 minutes each test), cheap, and at least as sensitive as conventional microscopy [2].

3. SEROLOGICAL MALARIA TESTS

Serological malaria tests may be beneficial in various conditions but are not indicated for the acute diagnosis of malaria. They include retrospective malaria diagnostics, chronic malaria screening, and blood banks’ testing of potential donors in previously unimmunatic individuals. Serological IgG response takes place quickly and usually within one week of parasitemia. After around one month and for several months to years, antibodies continue to worsen. The serologic tests for malaria in the immunofluorescence antibody trial (IFA) and malaria enzymes immunoassays (ELIA) have become a dependable one. It is incredibly sensitive and specific, yet it takes time and is subjective on the other hand. The IFA and 2 trade ELIA kits, Cellabs Pan Malaria Antibody CELISA and the Newmarket Malaria ELIA, were compared by She et al. [46] Cellabs ELIA detects IgG antibodies from P. falciparum, P. vivax, P. ovale and P. malariae collected by recombinant antigens. Newmarket malaria ELIA identifies IgG, IgM, and IgA antibodies using recombinant P falciparum and P vivax merozoite-specific proteins. The results for blood bank screening have been assessed with satisfactory results.

An immune test for Bio-Rad Malaria enzyme uses horseradish peroxidase (HRP) conjugated Plasmodium proteins to detect antigen – antibody complexes, which can also detect IgG, IgM, and IgA against P. falciparum and P. vivax. Cross-reaction with antibodies to P. malariae and P. ovale can also be observed [21].

4. POLYMERASE CHAIN REACTION (PCR)

Recently, some new malaria diagnostic methods have been developed as laboratory techniques based on flow cytometry and real time PCR [39] PCR has proven to be a useful diagnostic tool for the acute environment and can be made available more generally (Moody, 2002). At the erythrocyte stage, PCR assays are aimed at individual genes or parasite transcriptomes. Targeted genes or RNA transcriptions have been carefully evaluated and some recent developments in nucleic acid-based approaches [47].

The most flexible and accurate method for diagnosing malaria is PCR [34] PCR is a highly sensitive modality for molecular diagnosis that can identify asymptomatic, submicroscopic infection [48]. PCR restriction fragment length polymorphism (PCR-RFLP), random amplification of polymorphic DNA (RAPD), amplification fragment length polymorphism (AFLP), high-resolution melt curve (HRM) analysis, multiplex ligation-dependent probe amplification (MLPA), loop-mediated isothermal amplification (LAMP), and D (Sanger and next generation sequencing) [49]. To attain greater sensitivity and specificity than microscopic inspection, nested polymerase chain reaction (nested PCR) and quantitative PCR have been created. However, the need for relatively expensive equipment has hampered their deployment in field clinics [50].

Loop-mediated isothermal amplification LAMP method uses DNA polymerase as well as a set of four primers that are specifically tailored to identify six different target DNA sections. In one isothermal step, amplification and detection of the target gene can be completed [51,52]. In addition, Loop-mediated isothermal amplification is used to diagnose P. falciparum and P. vivax infections and detect asymptomatic malaria infections [53].
The Digital PCR dPCR Assays can be a valuable tool for evaluating very low human Plasmodium parasitemia. By employing quantitative polymerase chain reaction (qPCR) of massive blood volumes with DNA, the sensitivity of PCR to low-level parasitemia has increased. 12 whole blood samples must be processed simultaneously, without preliminary DNA extraction. The test is designed to target an RNA (rRNA) 18S Ribosomal gene that identifies all species of Plasmodium [48]. Other techniques use quantitative nucleic acid sequence amplification to identify all (r RNA)-based parasites and reliably detect mature gametocytes utilizing standard Pfs25 messenger RNA (mRNA) variations to detect all (r RNA)-based parasites. This is presently the most effective gametocyte detection diagnostic test available [54].

The Digital PCR is a feasible alternative. For malaria screening and diagnosis, dPCR should be investigated to supplement traditional testing [55]. According to studies, dPCR can provide a more precise quantitative parasite of absolute density than microscopy [52]. Furthermore, it was feasible to determine the duplex species specific method in samples where qPCR could not be utilized, as well as in minority of P. Malariae in mixed infections that were not recognized by qPCR in certain situations. These findings suggest that dPCR might be the method of choice for determining parasitaemia in asymptomatic and low-density infections. The accuracy of dPCR technology is being researched in order to enhance sensitivity, specificity, and test performance while assessing prospective blocking techniques. Wang et al. [56] used dPCR technology as a reference absolute quantification technique to assess a new semi-automated Taqman qPCR assay to identify single oocyst infections of the P. falciparum midgut microscope and the quantitative load of midgut parasite in the mosquito. dPCR has been shown in studies to be an absolute quantitative, reliable, and sensitive method. A dPCR test might be a useful tool for learning more about anti-malarial processes and demonstrating novel drug resistance mechanisms. LaMonte et al. [57] shown that dPCR may be used to investigate anti-malarial resistance mutations as an alternative to traditional PCR and DNA sequencing. Resistance to a range of anti-malarial medicines is found in P. falciparum pfcarl gene mutations, and different pfcarl mutations impart different levels of pharmacological resistance.

However, these alternative approaches (fluorescence microscopy and nucleic-acid techniques) are more expensive, need trained people, a high degree of complexity, and take several hours to detect malaria parasites. These approaches are not appropriate for use in rural and underserved healthcare settings. Flow cytometry also has a rather low detection limit. To stop malaria from spreading throughout the world, it's critical to develop a sensitive, accurate, and simple diagnostic technique for early detection [58].

5. MICROCHIP TECHNOLOGIES

This gap and restrictions require the development of additional diagnostic methods with better sensitivities. Simplicity, stability, and economic efficiency are all ensured. Chip-based microfluidics and biosensors were among the latest advancements [59].

Microchip technologies were supposed to enable high-throughput and extremely sensitive investigation of individual cell activities [60]. Previously, researchers developed a wonderful screening and analysis system for detecting malaria-infected erythrocytes from malaria cultures using a polystyrene cell microarray chip with 20,944 individually addressable microchambers, which allowed for ultra-high sensitivity and quick results [61].

The purpose of this cell microarray chip was to allow uniform dispersion of an erythrocyte suspension in a nucleus-staining fluorescence dye in microchambers, forming a monolayer, and analysis with a commercially available DNA microarray scanner to detect fluorescence-positive malaria nuclei in the erythrocytes. However, separating erythrocytes from malaria cells and identifying erythrocytes infected with fluorescent-positive malaria required the use of a centrifuge and an expensive DNA microarray scanner. Instead of a micronutrient centrifugation scanner and a DNA microarray, a push column with silicone oxide (SiO2) nanofibers was used to use a cell microarray chip system for medical application to separate erythrocyte from whole blood and a fluorescence detector with a CCD camera. In the past, the quantity of fluorescence and the shape of the bright spot were utilized to distinguish infected erythrocytes from uninfected erythrocytes. The fluorescence characteristics of individual erythrocytes were determined using the image processing program. [the average number of erythrocytes in microchamber 187
The new microarray chip technique can also be used to identify quantitative malaria as well as track single-cell malaria. A microarray cell chip system is used for reliable, highly sensitive, and fast detection of malaria parasites by employing a push column for clinical diagnosis and a high accuracy drug monitoring system for quantitative detection of malaria parasites [40].

Treatment: From the standpoint of public health, treatment is intended to reduce transmission to others by reducing the infectious reservoir and avoiding resistance to anti-malarial [62,63]. The fundamental goal of therapy is to eliminate parasites from a patient's bloodstream as quickly as possible to prevent an uncomplicated case of malaria from developing to serious illness or death. Effective treatment also decreases the spread of illness to others by reducing the establishment and spread of anti-malarial drug resistance. In the absence of an effective vaccine, therapeutic usage is the sole option for malarial disease treatment and prevention [64]. Malaria can be a serious, potentially death-based illness, and treatment should start as soon as feasible (particularly if it is caused by *P. falciparum*). Presumptive therapy should be begun regardless of the laboratory test findings if the patient exhibits signs or symptoms of severe malaria, and the laboratory test results are not available for more than 2 hours [1].

The majority of anti-malarial drugs presently target the asexual phase of malaria infection, which produces symptoms. The pre-erythrocytic (hepatic) stage is unappealing since it does not produce clinical signs. Anti-malarial medicines are highly selective in their phases (Burrows et al., 2014). The choice and treatment route mainly depends on the severity of the illness, which is often misunderstood and malaria species [65].

There were three primary groups of anti-malarial present: derived quinoline, antifolate and derivatives of artemisinin. The combination of medications that are often used at the same time to combat malaria infection efficiently also, medication combination reduces drug resistance and adverse effects [66]. As a result, practitioners have mostly employed artemisinin-based combination therapy (ACT) as a first-line treatment in the previous 10 years. ACT has just been approved as the first-line therapy for uncomplicated malaria caused by *Plasmodium falciparum* and *Plasmodium vivax* parasites that are chloroquine-resistant.

**Chloroquine:** Treat *P. vivax*, *P. ovale*, *P. malariae*, or *P. knowlesi* with chloroquine or ACT in areas where chloroquine infection is a concern. Treat uncomplicated *P. vivax*, *P. ovale*, *P. malariae*, or *P. knowlesi* malaria with ACT in chloroquine-resistant areas. Rapid recurrence in *P. vivax* or *P. ovale* with 14-day primaquine at 30 mg daily (except in pregnant women, women breastfeeding infants less than 6 months, and in G6PD deficient individuals). Consider administering primaquine base 45 mg once a week for eight weeks to patients with G6PD deficiency, under close medical monitoring to avoid primaquine hemolysis [67,68]. The combinations of sulfadoxine-pyrimethamine (SP) have become increasingly safe and cheap for the treatment of chloroquine resistant malaria. Nevertheless, increased use led to an increase in SP resistance. The resistance to *plasmodium falciparum* mainly describes point mutations in DHPS and DHFR SP targeted enzymes [69].

**Artemether:** Artemether is an effective artemisinin-based drug but is available for intramuscular injection only as a pre-mixed oil-based solution. Artemether is now widespread in many African countries, although not especially recommended by the WHO [70]. Atovaquone is the first anti-malarial medication that has been approved to target *Plasmodium* mitochondria. By blocking cytochrome b portions of a cytochrome bc1complex, by acting as a ubiquinone analog, atovaquone blocks electron transport. Atovaquone is safe and effective for pregnant women and children when used in combination with proguanil. Atovaquone is effective against both the parasite's host and mosquito sexual stages. As a result, the transfer of malaria from mosquito to man is limited. Malarone, a combination with proguanil, has been developed [71].

The WHO suggested that the successful use of anti-malarial medicines should be monitored annually by national malaria control programs to ensure effectiveness of the chosen treatments [65]. Overall, scientific researchers are forced to explore and develop new anti-malarial medicines by the frequently occurring anti-malarial drug resistance, including combinational therapies (CTs) regarding the high death rate and...
morbidity due to malaria and the inefficiency and urgency of available anti-malarial medications. Nevertheless, the discovery of new biochemical ways to generate malarial agents offers new chances to the new therapeutic medicines. The hunt for new cellular targets and the development of new therapeutic medicines are essential factors in the fight against drug-resistant malaria globally. The future development of anti-malarial will target medications more effectively with a particular action mechanism [17,72].

Malaria parasites have developed resistance to all major anti-malarial medication classes. In *P. falciparum*, the capacity to easily cultivate *P. falciparum* and progress in sequence technology and gene editing has substantially enhanced the ability to comprehend the impact of the mutations and confirm those alterations. These results have directly affected the assessment and monitoring of anti-malarial resistance and enable a detailed examination of why specific treatments fail and the invention of more effective therapies to treat anti-malarial diseases [66]. Entire *P. falciparum* and *P. vivax* genome scans using technologies, including in vitro and clinical research, such as microarrays and whole-genome sequencing (WGS), have offered insight into resistance mechanisms. Studies of genome-wide association (GWASs) have helped to find resistance genes. Important resistance mechanisms include point mutations or amplification of transporters' expressing genes, which are used for transporting drug to or from the digestive vacuole (DV) of the parasite. Mutations in transporters of the DV membrane producing genes are mainly mediating resistance to 4-aminoquinolin and aryl-amino-alcoholics. Resistance to antifolate and atovaquone mostly is caused by point mutations in the genes that code target enzymes that cause lower drug binding, as recently observed with the finding of kelch 13 mutations as an indicator of artemisinin resistance [66].

A new treatment regimen would boost its usefulness and longevity to prevent malaria at various phases of the life cycle. Based on the main metabolic differences between host and *Plasmodium*, the currently available anti-malarial drugs were identified. Some of the new drug design topics include heme detoxification, fatty acid synthesis, nucleic acid synthesis, and oxidative stress, which are some of the pest's primary metabolic activities [73].

The parasite's many enzymes, ion channels, transporters, and associated red blood cell (RBC) components, as well as oxidative stress molecules, lipid metabolism, and hemoglobin degradation, are all promising novel targets for creating new anti-malarial drugs [74].

Because malaria protease gene disruption impacts hemoglobin breakdown and erythrocyte production, malaria proteases are attractive treatment targets. This revealed that a protease plays a crucial role in parasite invasion of erythrocytes [75]. The crucial aspartic proteases *P. falciparum*, plasmepsins IX and X (PMIX and PMX) are promising targets in antimalarial areas because the inhibitors hinder egress, invasion and prevent rhoptry- and micronemal protein maturation that this process requires [76].

Favuzza et al. [77] discovered dual inhibitors of PMIX and PMX, namely WM382, that block several phases of the Plasmodium life cycle and showed that PMX is a master regulator of merozoite invasion and directs the maturation of proteins necessary for parasite growth, invasion, and egress. Oral treatment of WM382 cured mice of P. berghei infection and prevented hepatic blood infection. WM382 was also effective against *P. falciparum* asexual infection in humanized mice and inhibited mosquito transmission. The aspartic proteases Plasmepsin IX and X (PMIX and PMX) were shown to be potential candidates for malaria therapy and prevention.

The plasmodium species require enough substrate to sustain their healthy metabolism for rapid growth in infected erythrocyte. The parasite thereby prepares the host erythrocyte by generating specialist transport systems that are substantially different for the use and removal of metabolites than host cell transporters. The Plasmodial Surface Anion Channel (PSAC) is the most promiscuous target for intracellular parasite injections in the plasmodial surface anion channel (PSAC) and might be a viable target for the creation of a novel medication due to its critical involvement in various forms of nutrient acquisition [78].

Pyrvalate is metabolized to lactate to produce ATPs that must be reproduced inside the intraerythrocytic compartment. Glucose was first carried into the parasite erythrocyte via a GLUT1 glucose transmission combination found in the erythrocyte membrane and parasite-produced 'New Permeability Pathways. Hexose transports
glucose into the parasite via *P. Falciparum* (PFHT). Proposed that a possible fresh goal to produce a new anti-malarial medication is selective PFHT inhibition [79-80]. Lactate and glucose exports are essential for the maintenance of energy requirements, intracellular physiology, and parasite osmotic stability. The lactate is also a possible potential objective for the development of a new drug: H+ transport system inhibition [81].

Erythrocytes, like other cell types, have a low inner Na level. The parasite, on the other hand, increases erythrocyte cell membrane permeability, enabling Na to enter and boosting Na exit to extracellular levels. Thus the parasite exists in the intracellular location. In order to maintain a low cytoplasmic Na+ level for survival, it must transit Na+ ions across its plasma membrane. The Na+ influx is, in this case, regulated by a P-type ATPase (PfATP4) transporter, which is the major Na+ pump mechanism of the parasite. This inhibition generates a rise in Na+ inside the parasite, leading eventually to malaria parasite death [82]. The malaria parasite uses a complementary V-type ATPase transporter to regulate the increased H+ level and maintain an intracellular pH of 7.3. The aim is to produce a novel medication as the mechanism for action through mutant selection and genome sequence by inhibiting the V-Type H + ATPase [83].

Parasites need choline as a precursor to make phosphatidylcholine from scratch, which is essential for parasite development and survival. The parasite’s phosphatidylcholine production is hampered when choline is inhibited, resulting in parasite mortality [84] Albitiazolium is a Phase II medication, which works mainly by preventing choline from being transported to the parasite [85].

Heparan sulphate is used by the falciparum parasite in endothelium and other blood cells to restrict blood flow. Blood flow is restored, and the parasite’s growth is influenced by inhibiting such aberrant cells and harmful medication interactions [86]. Various studies have found that sevuparin is an anti-adhesive heparin polysaccharide with the abolition of antithrombin [87].

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**Fig. 2.** The *Plasmodium* parasite’s life cycle in humans, different types of anti-malarial drugs work at different stages and forms of the parasite [88]
Molecular biology engineering has established new multifunctional systems with outstanding qualities, such as increased sensitivity, specificity, and recognition. The word "nano" means "small or dwarf" in Greek. Nanoparticles (NPs) may also be known as particles, ranging from 1 to 100 nm in either direction or up to several 100 nm. And nano-biotechnology is both engineering and a molecular biology combination. Nano artifacts that include nanotubes, nanochannels, NPs, and nanotechnology have revolutionized the world [89].

The application of nano-biotechnology for patient therapy and vector combat may enhance malaria control in endemic areas. Successfully used compounds include lipids, proteins, nucleic acid, and metallic nanoparticles. Nano-biotechnologies are the best way to combat conditions of parasites and intend to eliminate malaria by giving appropriate treatment options and specifically aiming at parasite elimination [89,90].

Biogenic synthesis, which uses various biological materials of metallic NP such as silver, golden copper, and zinc, can potentially prevent malaria among different Plasmodium species. Green NPs including silver, palladium and platinum have been shown to handle the malaria parasites effectively. Bio-synthesized silver is critical to overcoming the production of malaria [91].

The biologically synthesized NPs benefit enormously from other approaches. The toxicity of drug molecules can be reduced by using nanotechnology. The AgNPs synthesis is the most efficient, environmentally friendly and economical process [89]. Chloroquine phosphate anti-malarial drug is encapsulated in gelatinous NPs and swelling regulated supplies in the physiological medium (pH 7.4). The goal of cerebral malaria CM therapy entering the brain was to investigate transferrin-conjugated solid lipid NPs (SLNs). Biological anti-plasmodial properties have currently been validated in vitro and in vivo. The brain consumption of quinine was markedly higher than unconjugated SLNs or drug solutions [92,93]. Artemether liposomes with 24 hours low-frequency and encapsulated beta artemether for malaria-resistant treatments are more efficient and easily accessible at the target area for reducing dose size [94].

Prevention: Expanded access to WHO-recommended malaria prevention methods and tactics, such as effective vector control and the use of preventative anti-malarial medicines, has significantly reduced the worldwide burden of the illness since 2000 [1].

Malaria can also be prevented using the ABCD method. Which stands for Risk Awareness - Find out whether you're at risk of malaria. Bite prevention - Use insect repellent, cover your arms and legs, and use a mosquito net to avoid mosquito bites. Check if you require malaria prevention tablets, and if so, ensure that you take the necessary anti-malarial medicine tablets at the exact dose and complete the course. Diagnosis - if you have symptoms of malaria, get medical help right once, even if it's been a year since you traveled [95–98].

6. EFFECTIVE CHEMOPROPHYLAXIS

Malarone (Atovaquone-proguanil), is one of the most effective prophylactic agents for malaria its effectiveness comes from its good tolerability and liver level, which only requires one more week of post-exposure care for a recommendation with a low potential for serious adverse effects [21]. Atovaquone-proguanil inhibits the P. falciparum blood stage and its liver process, for which activity causal prophylaxis is considered for this pathogen that reduce the need for weeks of prophylaxis after the flight, which are essential to blood stadium agents such as doxycycline or mefloquine [97].

Doxycycline, mefloquine, CQ and PMQ are alternative chemoprophylaxis agents recommended for passengers. Research have shown a high efficacy of doxycycline against Plasmodium variable infection and relatively low levels of adverse events comparable with atovaquone-proguanil [96,99].

Primaquine is now recommended by the World Health Organization as a transmission-blocking medication, although its usage is limited in some populations due to toxicity. As a result, new, safe, and therapeutically effective transmission-blocking medicines must be found. Natural products have been widely researched in the development of chemotherapeutic anti-malarial drugs. The effects of natural products (and their derivatives) derived from plant and microbial sources on sexual stages of Plasmodium parasites and the Anopheles mosquito vector were investigated, as well as the challenges and opportunities that exist and how they can be mitigated and/or exploited in order to accelerate
the development of transmission-blocking drugs derived from natural products [100].

**Vaccinations:** In addition to novel drug discoveries, the effective development of vaccines is essential to the fight against malaria [101]. In the last few decades, several efforts have been made to develop efficient and inexpensive preventive vaccines against malaria in the least possible doses [102].

Several candidates have recently been identified for the malarial vaccine, and with the advent of new biotechnologies, genetic manipulation is being made more successful [103]. In clinical studies, malaria vaccine candidates have advanced. To expand or strengthen our existing candidate portfolio, scientists are focusing on antigen discovery, structural vaccination studies, and better platforms. As the portfolio grows, more resources will be necessary to create prospective candidates; as more candidates progress to products [104].

Antigens produced throughout the pre-erythrocytic, blood-stage, and mosquito-sexual stages of the process might be utilized in malaria vaccines or a "vaccine to interrupt malaria transmission" (VIMT) to limit parasite transmission in the population. The candidates for malaria vaccination are categorized according to the stage of the *Plasmodium* life cycle in which the targeted antigen is expressed [104–106].

Infection does not provide total immunity to people of endemic places, but it allows for some acquired immunity, resulting in less severe future malaria episodes. The development of vaccines from different phases of the *Plasmodium* life cycle was the focus of research to provide more positive protection. Accomplished vaccines were intended to target the various stages of the parasite sporozoites; hepatic stages (merozoites, schizonts, and hypnozoites) and erythrocytic stages (rings, trophozoites, schizonts, gametocytes). Accordingly, three main types of vaccines were considered for targeting the different stages of the parasites’ life cycle: vaccines for pre-erythrocytic stages to decrease the probability of infection; vaccines for erythrocytic stages to control the infection and minimize pathological effects; vaccines to block transmission and prevent infected mosquitoes from spreading the disease; it could minimize malaria burden and mortality in Africa and in other parts of the world's most malarial continent [107]. The goals of vaccine development were four phases of the malaria parasite’s life cycle (Fig. 3) as Pre-erythrocytic stages (radiated and genetically attenuated and recombinant subunit sporozoites); blood stages (chemically attenuated liver-stage and blood-stages, subunit merozoite protein and merozoites adhesin required for erythrocyte invasion); and mosquito stages (pre-fertilization and post-fertilization subunit antigens, and subunit compatible sequences proteins that allow the parasite to evade the Anopheles gambiae immune system) [104].

The multi-stage vaccine included: two sporozoites antigens, the circumsporozoite protein CSP and the sporozoite surface protein 2 (PISSP2); one from the liver stage (LSA1); three from the erythrocytic stages (merozoite surface protein 1, serine repeat antigen, and an apical membrane antigen, AMA1); and one sexual stage antigen (25-kDa Pfs25). While experimentally, the CSP, PISSP2, MSP1, Pfs25 antigens elicited prominent specific cell-mediated immune responses in humans, the antibody responses were poor [108].

The circumsporozoite protein (CSP), which is involved in sporozoite movement and invasion, was the first malaria gene to be cloned. It is found on the surfaces of sporozoites and early exo-erythrocytic hepatic stages, creating a thick sheath around the parasite. Several times, it has been tried as a potential vaccination candidate for inducing meaningful protective immune responses [109]. Additionally, a vaccine based on a recombinant CSP, covalently bound to a purified *Pseudomonas aeruginosa* toxin (A9), was found to produce antibodies that prevent invasion of hepatocytes and a cellular response from enabling the destruction of infected hepatocytes [110].

Vaccines targeting blood-stage forms such as apical membrane antigen-1 (AMA-1) and merozoite surface protein 1 (MSP1) have had limited success due to the complex metabolic processes of erythrocytes and merozoites [101,111].

A promising blood-stage candidate for the antigen included in a broadly reactive malaria vaccine is the *Plasmodium falciparum* (Pf)-rich cysteine-protective antigen (PFCyRPA). The protein is well preserved among several geographical strains, and it can efficiently inhibit the entry of malaria parasites into red blood cells. It plays an essential part in the red blood cell
invasion process of *P. falciparum* merozoites and PfCyRPA antibodies. Recombinant PfCyRPA is chemically combined with phosphoethanolamine and incorporated as an antigen supply system for humans into the membrane of unadjuvanted influenza virosomes. To assess its immunogenicity and its ability to elicit binding parasites and growth-inhibiting antibodies, Laboratory animals were inoculated with the virosome-based PfCyRPA vaccine, the vaccine produced high titers for PfCyRPA-specific antibodies linked to the parasites in the blood stage. Therefore, Influenza virosomes provide an appropriate antigen supply mechanism for inducing protective against recombinant PfCyRPA antibodies and are designated as a suitable component to be included in a multivalent and multi-stage virosome malaria vaccine [112].

The most promising strategy is to develop vaccines that target the pre-erythrocytic sporozoite stage. Anti-circumsporozoite antibody is induced by the RTS, S/AS02A vaccine, which has completed Phase III effectiveness studies [101]. This was a major turning point in vaccine research since it was the first human parasite vaccine to pass the most stringent regulatory inspection (dubbed WHO-listed authority maturity level 4 (WLA ML4)), which serves as the gold standard for all subsequent vaccinations. RTS, S/AS01E pilot implementation initiatives were begun in 2019 in response to a request from the WHO to investigate the safety and advantages of administration via normal public health channels [38,104]. The findings will be analyzed by international organizations like as the WHO and RTS once the ongoing implementation projects in three African countries are finished in 2022, and S/AS01E will be appraised for wider use in Africa by national policymakers [91,104].

Recent trials have yielded a successful malaria vaccine and highlighted two malaria vaccines with promising outcomes: a *P. falciparum* sporozoites PISPZ vaccine made by Sanaria Inc., and a recombinant fusion proteins formula (RTS,S/AS01) made by GlaxoSmithKline (GSK), commercially known as ‘Mosquirix’ [107].

While RTS, S lowers clinical malaria in African children, innovative pre-erythrocytic vaccine (PEV) alternatives like as R21/Matrix M and PISPZ whole sporozoites vaccines (WSV) full-length CSP immunogens are being developed to increase effectiveness. Throughout the last decade, transmission-blocking vaccinations TBV have proceeded to Phase 2 clinical trials. To achieve malaria eradication, efficient TBVs that target parasite sexual stages can be employed in conjunction with existing malaria management techniques, as well as the most effective pre-erythrocytic vaccines. Transmission-blocking (killing mosquito/sexual stage parasites) vaccines, for example, PpPf S25 including a field experiment and the first generation of placental malaria vaccine, were tried in the previous decade (that clearly contains placental parasites) [104]. Placental malaria vaccines PMVs are a kind of blood-stage vaccines BSV that targets antigens on the surface of infected erythrocytes, causing placental malaria by sequestering in intervillous gaps. The immunodominant PIEMP1 variant surface antigen family, which enhances parasite sequestration and hence *P. falciparum* pathogenicity, and is a target of naturally acquired protective antibodies, is one of these [113]. VAR2CSA is a well-organized member of the PIEMP1 family, which the parasite uses to sequester itself in the placenta. These vaccines may be capable of providing functional activity against homologous parasites, according to preliminary studies [104].

BSV, which targets blood-stage merozoites invasion proteins and can limit blood-stage multiplication or abort infection during the blood stage, has had dismal results in human studies over the previous two decades. The creation of novel or better immunogens that target non-redundant merozoite invasion pathways might assist to improve these dreadful results. In the meanwhile, vaccines targeting several BSV targets, such as infected red cell surface proteins, schizont egress antigens, and attenuated infected erythrocytes, are being tested in preclinical and clinical studies [104].

The activation of programmed cell death of intraerythrocytic trophozoites in vitro and naturally acquired PIGARP antibodies were linked to the control of *P. falciparum* parasitemia and severe malaria protection. PIGARP has recently been discovered as a protective antibody target for a non-infected erythrocyte surface protein. The erythrocyte parasite's egress has also been discovered as a target for anti-*P. falciparum* Egress Antigen 1 Schizont antibodies (PISEA-1) [104,114].

While a highly efficient vaccine is the goal of much fundamental malaria research, it is clear how challenging such a goal is due to the absence of a sterilizing immune response to
naturally acquired disease. Current candidate vaccines offer 30–50 percent protection effectiveness [115].

A new finding of antigens is promising to improve existing vaccination candidates, human monoclonal antibodies (104). Monoclonal antibodies (mAbs) inhibiting the CSP map to either a shortened cross sequence or a repetitive core region (NPNA). Scientists evaluated in vitro and in vivo six CSP-specific mAbs of human vaccination recipients RTS, S/AS01 (mAbs 317 and 311); complete sporozoite vaccine PfsPZ (mAbs CIS43 and MGG4); or malaria exposed patients; (mAbs 580 and 663). The strongest affinity was RTS, S mAb317 that specifically binds the epitope (NPNA). MAb CIS43, which has double-specific binding on the joint sequence and NPNA, was the most powerful inhibitor of in vitro sporozoites intrusion. In vivo protection was also connected with the buried surface between mAb and its target epitope. In vitro-in vivo readings and disconnections have substantial consequences for the design and downsizing of CSP-based therapies of the next generation [116].

To induce more potent humoral and cell-mediated immune responses, relevant approaches call for consideration of DNA based vaccines as a source of maintained protein expression of multiple antigens from both pre-erythrocytic and erythrocytic stages. Several DNA vaccines were effective in animal models and are consequently undergoing clinical trials [110].

**Measurements against mosquitoes:** The capacity to prevent *Plasmodium* parasite transmission from humans to mosquitoes and mosquitoes to humans is critical to meeting the ambitious goal of eliminating malaria [100]. The use of Indoor residual spraying (IRS) such as DDT and other insecticides initially destroys female malaria-related mosquitoes. However, the use of these insecticides has significantly reduced the annual parasite index (API) worldwide. Reduced API forces the WHO to develop and implement different control strategies [90] Insecticide-treated nets (ITN) are a commonly used method that has shown efficacy in malaria prevention and control in countries endemic to malaria. However, households differ widely in the use of ITNs as a control tool for malaria transmission [118].

To alleviate the growing problem of mosquito-borne diseases, better and more effective gene editing methods are needed. The targeted disorder of genes in mosquito vectors of human infections is a potent way to detect and develop innovative ways to mosquito disease management and biology of mosquito pathogen transmission [2,119].

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![Fig. 3. *Plasmodium* life cycle phases and vaccine options that target each step This picture was derived from one that was originally published by [117] and more current malaria vaccine candidates have been added to the list [104]](image-url)
The legacy of a refractory gene may be used to establish a vector population unable to transmit illness or disrupt vital genes for viability or fertility that may ultimately eradicate a population. These methods, known as population substitution and population suppression, aim to reduce or may eliminate specific pathogenic mosquitoes in general. There are several types of localized and non-localized drives with distinctive properties that impact performance, safety, and regulation. In contrast, the only local population will be affected by local drives and, in some situations, will be excluded over time. This may provide significant issues for regulators and stakeholders, if gene drives move beyond political limits into ecologically sensitive regions or locations with opposing technologies [120].

Marshall and Akbari [121] Raban et al. [120], analyzed the many types of gene drive systems. Unlocalized drives will spread beyond a release location and will remain for many generations in the population.

With mosquito demonstrations, recent development in gene-editing technology, namely clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated protein 9 (Cas9)-based genome technology, has accelerated [122,123]. CRISPR/Cas9-based gene disruption is a valuable tool for learning about the underlying biology of vector-pathogen interactions, and it may also be used to develop mosquito control methods. While several CRISPR/ Cas9 approaches have advanced mosquito genetics research, these techniques rely on embryo injection of the Cas9 ribonucleoprotein complex (RNP), which necessitates specialized and costly equipment and training. To circumvent this limitation, ReMOT Control (Receptor-Mediated Ovary Transduction of Cargo) was created to transport gene-editing moieties to the arthropod germline from the hemocoel, allowing targeted and heritable alterations to be produced by adult injection rather than embryo microinjection [124].

Receptor-Mediated Ovary Transduction of Cargo Control was tested in Aedes aegypti, but the method's applicability to more resistant to editing species (such as Anopheles mosquitoes) has yet to be determined. Embryo injection techniques for genetically modifying mosquitoes (primarily Anopheles) are, nevertheless, difficult, and ineffective, especially in non-specialist institutions. The application of ReMOT Control to Anopheles extends the power of CRISPR/Cas9 techniques to malaria laboratories without the technology or knowledge to do embryo injections, as well as demonstrating ReMOT Control's versatility across mosquito species. Adult mosquito ovaries were used to modify the ReMOT Control method for delivering Cas9 ribonucleoprotein complex, resulting in targeted and heritable mutations in the malaria vector Anopheles stephensi without the use of embryos. ReMOT Control gene editing was equally effective as conventional embryo injections in Anopheles [125].

Huang et al. [126], revealed that using mosquito microbiota has tremendous promise for reducing mosquito-borne disease transmission and highlighted the problems of eventually releasing genetically modified (GM) symbionts in the field. The mosquito microbiota is derived from the environment, and its composition is very variable, depending on species, diet, development stage, and geographic location. The midgut and, to a lesser extent, the salivary glands and reproductive organs are colonized by microbiota. The mosquito microbiota is involved in many aspects of the host, including feeding, digestion, mating, sexual reproduction, development, immunological function, and disease resistance. Microbiota, whether GM or not, has been recommended for mosquito population control and illness prevention. In the field, the introduction of GM symbionts designed to generate antipathogen compounds in mosquitoes yielded excellent results.

7. CONCLUDING REMARKS

Malaria remains a persistent threat in recent research. At the beginning of the 20th century, scientists tried to describe a successful way of eradicating malaria. There is no single control mechanism effective in the battle against malaria, so many strategies must be combined to achieve convergence in total controls and the ultimate eradication of diseases. Consequently, eradication of malaria depends mainly on four strategies: 1) rapid diagnosis, 2) effective chemotherapy (Anti-malarial drugs), 3) control transmission by chemoprophylaxis and vaccination, and 4) measurements taken against mosquitoes. New strategies for malarial eradication included:

- Diagnosis by an ultra-sensitive rapid diagnostic and Digital PCR.
- Treatment: Novel drug targets.
- Recent vaccine trials.
Mosquitoes Gene editing and the use of mosquito microbiome have showed significant promise in reducing mosquito-borne disease transmission.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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