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Review Article

# Drugs Resistance Gene Mutation (MDR1) of *Plasmodium falciparum*

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**Abstract:** Malaria ranks among the most significant health threats, alongside human immunodeficiency virus (HIV) and tuberculosis influenza. In 2004, Sudan implemented artesunate combined with sulfadoxine/pyrimethamine (SP) as the primary treatment due to the notable resistance of falciparum to antimalarial drugs. The effectiveness of artemisinin combination therapies depends on both artemisinin and the partner drugs. Changes in the Pfmdr1 gene have been associated with chloroquine resistance in specific *Plasmodium falciparum* isolates. Variations in the *Plasmodium falciparum* chloroquine resistance transporter (pfcrt) and *P. falciparum* multidrug resistance 1 (pfmdr1) genes are linked to reduced sensitivity to amodiaquine and lumefantrine. However, the exact impact of these variations on treatment outcomes with artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) remains unclear. Alterations in pfmdr1 are linked to decreased uptake of multiple anti-malarial medications, resulting in diminished intracellular levels. Single nucleotide polymorphisms (SNPs) in pfmdr1 correlate with resistance to aminoquinolines. This article examines the development of CQ resistance in Sudan, along with the evidence regarding the effectiveness of current first-line treatments. The situation in Sudan is compared with trends in other African nations and globally, aiming to clarify potential future outcomes for Sudan. Notably, Northern Sudan has been a key region where the initial signs of drug-resistant malaria parasites were identified in Africa.

**Keywords:** Malaria, Drug resistance, Sudan, Chloroquine, Artemisinin.

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## INTRODUCTION

Malaria has been one of the most severe diseases throughout human history, including the prehistoric era. It rivals influenza, tuberculosis, and the bubonic plague. As the most common protozoan illness, it continues to threaten 40% of the global population each year. The term comes from the Italian words "mal," meaning "bad," and "aria," meaning "air." Many victims contracted malaria after this type of exposure, leading medieval myths to suggest that the disease was caused by evil spirits or mists and vapors emanating from wetlands [1]. Today, we know that the primary habitat for the mosquito vector is a swamp. The four species of *Plasmodium—P. malariae, P. vivax, P. falciparum,* and *P. ovale*—are obligatory intracellular sporozoans that cause malaria. The main vertebrate hosts for these species, which vary in disease pattern and severity and are geographically distinct, are humans and certain monkeys. The female Anopheles mosquito is the primary vector for all forms of malaria, although transmission can also occur through shared needles, blood transfusions, and from mother to fetus [2]. Malaria presents a severe public health challenge in Sudan, with an estimated 7.5 million infections and 35,000 fatalities annually. A new antimalarial drug policy adopted by Sudanese health authorities in June 2004 recommends artemether-based combinations (ACT), with artesunate + sulphadoxine (ASP) as the first line of treatment and artemether/lumefantrine (A/L) as the second [3]. More than 95% of malaria cases in Sudan are caused by P. falciparum, and resistance to CQ and SP is well-documented [4]. The goal of ACT's widespread

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distribution in malaria-endemic areas is to prevent the development and spread of resistance [5]. In 2004, artemether-lumefantrine (AL) and artesunate/sulphadoxine-pyrimethamine (AS/SP) were defined as the first and second lines of treatment, respectively, for uncomplicated P. falciparum malaria. With some patients returning with recurrent infections, practicing physicians have recently questioned the effectiveness of AS/SP. Additionally, similarly to South East Asia, the emergence of slow-clearing parasites after artesunate treatment has led to heightened monitoring of ACT efficacy and the need for rigorous studies on molecular indicators of artemisinin and partner drug resistance [4]. Currently, there are only a few methods available for diagnosing malaria. Traditional techniques include using light microscopy to evaluate stained peripheral blood smears, empirical/syndromic diagnosis (primarily based on the presence of fever in endemic areas), and clinical diagnosis based on patient history and physical examination. Although useful in certain settings, histopathology plays a limited role and is not beneficial for malaria control initiatives. Similar to other prevalent infectious diseases, several rapid diagnostic tests (MRDTs) for malaria have been developed and brought to market. Of the 65 diagnostic methods currently available, they may have the greatest impact on malaria detection and treatment initiatives [6]. Good tolerance and safety (especially in young children), cost, availability in endemic countries, and brief treatment regimens are critical characteristics for the effective use of antimalarial medications. Nearly all antimalarial drugs are now administered as part of combination therapy, with each drug targeting different processes within the parasite to reduce the establishment of drug-resistant strains primarily [7]. Introduced in the late 1940s, chloroquine is a 4-aminoquinoline that is widely used to treat and prevent malaria. For many years, it was the gold standard for treating malaria due to its effectiveness, affordability, and safety—even during pregnancy [8]. Plasmodium resistance mechanisms depend on several factors, including the parasite's mutation rate, the fitness costs associated with resistance mutations, the overall parasite load, the effectiveness of medication selection, and treatment compliance [7]. Falciparum malaria, the leading cause of morbidity and mortality, is particularly challenging to manage across Africa and southern Asia due to resistance to anti-malarial medications. The first reports of P. falciparum chloroquine resistance (CQ) emerged near the Thai-Cambodian border in Southeast Asia in the late 1950s. Subsequent research indicated that neighboring countries in Asia, Africa, and South America also developed CQ resistance. Moreover, P. falciparum has shown resistance to several anti-malarial medications, including mefloquine, atovaquone, artemisinin, and a combination of pyrimethamine and sulphadoxine [9]. Historical evidence indicates that drug-resistant *P. falciparum* strains first surfaced in Southeast Asia before spreading to Africa. Drug resistance, which likely originated in Southeast Asia, has mainly spread and evolved in East Africa [10]. The 1990s witnessed an increase in malaria morbidity and mortality attributed to the spread of CQ resistance. In 2001, the World Health Organization (WHO) recommended treating uncomplicated *P. falciparum* malaria with artemisinin-based combination therapy (ACT) [11]. Generally speaking, compared to West Africa, resistance is more pronounced in East and Southern Africa.

**Epidemiology of malaria**

Unlike the epidemiology of malaria infection, epidemiologists have recently placed greater emphasis on the epidemiology of clinical malaria. The two most prevalent types of severe malaria in Africa, cerebral malaria and severe malarial anemia, exhibit distinct epidemiological patterns, according to research on the disease's epidemiology. Severe malarial anemia predominantly affects young children and is particularly prevalent in areas with extremely high malaria transmission. Conversely, cerebral malaria is more frequently observed in regions with low transmission, especially those that are seasonal, and it primarily impacts older children. Studies on patients with uncomplicated malaria have defined fever thresholds and established the relationship between fever and parasite density [12].

**Immunity**

The malaria parasite expresses a broad range of proteins at various stages of its complex, multi-stage life cycle, and these proteins also undergo frequent changes. As a result, a natural infection with malaria parasites only produces a temporary and incomplete immunity that cannot protect an individual from subsequent infections. The complex interaction between parasite proteins and the host's immune system has also made developing a vaccine to prevent the disease challenging, if not impossible, to date. There are two types of immunity to malaria: acquired or adaptive immunity and natural or innate immunity [13].

**Natural or innate immunity**

An innate refractoriness of the host that inhibits the formation of an infection or triggers an immediate inhibitory response against the entry of the parasite is known as innate immunity to malaria. The host's innate immunity is spontaneous and independent of any prior infections. Research has shown that changes in hemoglobin structure or specific enzymes protect against the infection or its severe symptoms; these traits are commonly observed in regions with high malaria transmission [14]. Red blood cells with Duffy negativity exhibit immunity to *P. vivax* infection. Due to its high prevalence in Africa, this parasite may have been nearly eradicated from the continent [15]. Certain thalassemias have been noted to protect against *P. falciparum* or *P. vivax*, resulting in a 50% reduction in infection, while homozygous hemoglobin C correlates with a 90% reduction. Other protective factors include hemoglobin E and ovalocytosis carrier status. According to Doolan *et*

*al*., (2009), sickle cell hemoglobin (90%) and glucose 6-phosphate dehydrogenase deficiency (50%) confer protection against severe malaria and related mortality [16]. Furthermore, an acute malarial infection triggers a rapid, non-specific immune response that tends to slow the disease's progression. The humoral and cellular processes of this "nonspecific" defense are not fully understood. Primary drivers of this response include primary extrathymic T cells (Natural Killer, or NK), intermediate toll-like receptor (TCRint) cells, and B-1 cells that produce autoantibodies. Natural killer (NK) cells are present in blood, secondary lymphoid organs, and peripheral non-lymphoid tissues. In vitro, NK cells have been shown to proliferate and lyse erythrocytes infected with *P. falciparum*. Peripheral blood NK cells respond to Plasmodium-infected erythrocytes by producing interferon-gamma, which activates parasiticidal macrophages. This response may be more crucial for innate malaria immunity than their ability to lyse infected host erythrocytes [17]. Additionally, these cells play a role in the formation and initiation of adaptive immune responses. The pro-inflammatory chemokine Interleukin-8, produced by NK cells, assists in activating and recruiting other cells during malaria infection. Gamma delta T cells, NKT cells, dendritic cells, and macrophages all detect the parasite and participate in the immune response. In vitro, NKT cells effectively inhibit liver-stage parasite replication in mouse malaria systems. Despite malaria infection leading to significantly increased blood concentrations of non-malaria-specific immunoglobulin, the importance of underlying polyclonal B-cell activation for innate immunity remains unclear [18].

**Acquired or adaptive immunity**

After infection, adaptive immunity to malaria develops, and its effectiveness in preventing malaria varies based on the host's characteristics, location, and the number of infections experienced. There is significant overlap among the three types of immunity, classified as anti-disease immunity, which protects against clinical disease; anti-parasite immunity, which safeguards against high parasitemia; and sterilizing immunity, which prevents new infections by maintaining low-grade, asymptomatic parasitemia, also known as premunition [19]. A non-immune person often experiences an acute clinical illness with extremely low levels of parasitemia after contracting malaria; the infection can then progress to severe illness and death. A few additional infections lead to the development of anti-disease immunity, reducing the risk of severe illness while suppressing clinical symptoms even in the presence of high parasitemia [20]. Anti-parasite immunity develops gradually as a result of repeated and frequent infections, resulting in very low or undetectable parasitemia. While sterilizing immunity is never completely achieved, it produces a high level of immune response, minimal parasitemia, and an asymptomatic carrier status. According to Doolan *et al*., (2009), premunition refers to immunity that is primarily mediated by the presence of parasites rather than by prior infections [16]. The emergence of clonal antigenic variation during an infection and the existence of genetically and antigenically distinct strains of the parasites in a specific location prompt the host to develop an immune response against these varying strains and antigenic variants [21]. Consequently, the development of immunity against malaria is notably slow, ineffective, and specific to strain and species. However, acquired immunity develops at a very early age in areas with high malaria transmission and stable endemic malaria, such as sub-Saharan Africa and forest regions in most Indian states. In these areas, maternal antibodies protect infants born to immune mothers from disease during the first six months of life. After this passive immunity, there exists a period of heightened sensitivity for one to two years before active immunity develops [16]. Depending on transmission rates, the risk of clinical disease increases from birth to approximately six months of age. From around three to four months, infants are at risk for severe illness and death. Children aged 2 to 4 are more susceptible to cerebral malaria as they grow older. Due to recurrent and frequent infections, the incidence of clinical disease starts to decline, and the risk of death significantly decreases between the ages of two and five. By adulthood, most residents typically have sterilizing immunity. In contrast, residents in unstable endemic regions usually only develop partial immunity [16]. As pattern-recognition receptors (PRRs) like Toll-like receptors (TLRs) and cluster of differentiation 36 (CD36), or inflammatory cytokines like interferon (IFN-γ) or dendritic cells (DCs) mature and migrate to the spleen—the primary site of immune responses against blood-stage Plasmodium parasites—the level of antimalarial immunity affects the clinical outcomes of the disease across various locations and age groups [16]. The upregulation of adhesion molecules, MHC class II molecules, CD40, CD80, and CD86, along with the generation of cytokines like interleukin-12 (IL-12), is associated with DC maturation [22]. IL-12 stimulates T helper 1 (TH1) cell development and activates natural killer (NK) cells to release IFN-γ. The production of cytokines, particularly IFN-γ, by NK cells facilitates the clonal proliferation of antigen-specific naive CD4+ T cells, leading to DC maturation and enhancing the effects of maturation stimuli derived from the parasites. The adaptive immune response is further enhanced by NK cells producing IFN-γ, which triggers DC maturation and activates macrophages, aided by IL-2 generated from antigen-specific TH1 cells. Both innate and adaptive responses are negatively regulated by cytokines, including transforming growth factor-β (TGF-β) and IL-10. The extracellular merozoites in circulation are the main targets of acquired immunity, which is primarily directed against the asexual erythrocytic stage. Protective immune responses also target the pre-erythrocytic stage, but they are ineffective in preventing sporozoite invasion or the parasite's intrahepatic development [16]. Malaria infection may trigger cell-mediated immune responses that offer protection against

pre-erythrocytic and erythrocytic parasite stages. In both human and murine malaria, CD4 T cells are crucial for immunological defense against asexual blood stages. The role of CD8 T cells in this context, which play a significant effector role in pre-erythrocytic immunity and help prevent severe malaria, remains unclear.

**Malaria diagnosis methods**

In many countries, laboratories struggle to diagnose malaria effectively. The most sensitive techniques for diagnosing malaria are often impractical for typical laboratory settings, as blood samples from patients suspected of having acute malaria must be examined urgently. Laboratories may lack the necessary microscopy expertise for malaria diagnosis, and factors such as endemic malaria, population shifts, and international travel all exacerbate the problem. Diagnostic challenges arise from changing patterns in the recognized morphological appearances of malaria species, which may stem from medication pressure, strain variation, or blood collection methods. Meanwhile, the World Health Organization (WHO) has initiated a dialogue on the practical possibilities of developing rapid, sensitive, and affordable diagnostic tests for malaria with scientists, physicians, and manufacturers of diagnostic equipment. These rapid assays must meet requirements such as detecting 100 parasites/µl across all Plasmodium species and enabling semi-quantitative assessments for monitoring drug therapy outcomes [23].

**Microscopy**

All malarial nations require reliable laboratory diagnostic services to ensure early and effective treatment, accurate epidemiological data, and epidemic preparedness, despite the differences in the fundamental goals of malaria control, which range from eradicating mortality and minimizing morbidity to reducing prevalence or achieving eradication. Giemsa microscopy is considered the best diagnostic tool for managing malaria because it is low-cost, can differentiate between various types of the disease, and can quantify parasites. Microscopy is now more feasible in remote locations thanks to solar battery chargers and high-quality light-emitting diode (LED) lighting. Nevertheless, microscopy requires skilled, trained microscopists, rigorous maintenance of operational infrastructures, and efficient quality assurance (QA) and quality control (QC). Microscopy was the primary method for diagnosing malaria during the Malaria Eradication (ME) period. Despite the logistical and organizational challenges, the disease was successfully eradicated in many countries, including 50% of the inhabitants of the original malarial regions. However, many nations, especially in the Americas and southern Asia, failed to establish or maintain the system, leading to a lack of attention to diagnostic services. I was never regarded as a realistic goal in tropical Africa. Due to the still-effective "presumptive treatment" at the time of blood sampling, countries with well-managed ME programs benefited from a time buffer between the collection and evaluation of blood samples from suspected malaria cases. Conversely, drug resistance became more widespread in the nations where I struggled, and most of them were ill-prepared to handle the new challenges posed by P. falciparum's resistance to 4-aminoquinolines and antifolates. In most affected nations, artemisinin-based combination therapy (ACT) is currently or will soon be the first-line drug for treating P. falciparum. Before administering ACT, which may be more expensive and less well-tolerated, an accurate diagnosis is deemed necessary. Demirev PA and colleagues (2002) discuss staining methods. Smears can be stained with various Romanowsky stains, including Field's, Giemsa's, Wright's, and Leishman's. For parasite screening, thick films are best stained with Giemsa's stain or the quick Field method [24]. A thick blood film has a sensitivity of 5–10 parasites/µl. In contrast, thin blood films stained with Giemsa's or Leishman's stain are useful for both parasite specificity and detecting stippling of infected red blood cells, with a sensitivity of 200 parasites/µl. The dye performs best at a pH of 7.2. Slides must be dry and clean [25]. Giemsa (Rapid) staining is applied after the thick film has been dehemoglobinized in water. Prepare a 10% Giemsa stain in pH 7.1 buffered water. Immerse the slide in the stain for five minutes. Rinse briefly in a jar of tap water for one or two seconds. Drain, pat dry, and inspect. For Standard Giemsa, prepare a 4% Giemsa in a pH 7.1 buffered solution. Soak the slide, which should be at least 12 hours old, in the stain for 30 minutes. After draining and rinsing with fresh water, drain again and inspect [26].

**Thick and Thin smear**

Newsprint is hardly visible through the heavy smear of the proper thickness. It is not fixed with methanol and is dried for half an hour. This makes it possible for the red blood cells to hemolyze, leaving only leukocytes and any malaria parasites that may be present. However, the sluggish drying and hemolysis can deform the plasmodia shape, which makes species separation challenging. Therefore, thick smears are employed to quantify parasite concentration and detect infection [24]. Give the thin smear ten minutes to air dry. The thin smear needs to be fixed in methanol after drying. This can be accomplished by dabbing the thin smear with a cotton ball soaked in methanol or by dipping it into methanol for five seconds. Every precaution should be made to prevent the thick smear from coming into contact with methanol while fixing the thin smear [24].

**Molecular methods**

Specifically, the 1980s and 1990s saw the introduction of polymerase chain reaction (PCR) and DNA probes. Fluorescence staining techniques for identifying malaria parasites also surfaced, such as flow cytometry, interference filter systems for acridine orange-stained thin blood smears, and quantitative buffy coat [QBC] analysis. In 1999, Hanscheid T. Depolarized laser light and mass spectrometry were used to select

malaria pigments, with varying degrees of effectiveness [27]. Malaria parasites can be found by using the non-isotopically labeled probe after PCR amplification. PCR-based studies have demonstrated good sensitivity and specificity for identifying all four species of malaria in travelers returning to industrialized nations, especially in cases of mixed infections and low-level parasitemia [28]. According to one study, the PCR test can identify 1.35 to 0.38 parasites/µL for P. falciparum and 0.12 parasites/µL for P. vivax, making it ten times more sensitive than microscopy. In cases of unexplained fever, the PCR test has also been found to be helpful in determining the diagnosis of malaria [24].

**Quantitative buffy coat (QBC)**

Becton and Dickinson Inc. developed the QBC test, an innovative method for detecting the malarial parasite in peripheral blood. It involves applying acridine orange dye to the compressed and centrifuged red cell layer and examining it under a UV lamp. Compared to the traditional thick smear test, it is quick, simple, and reportedly more sensitive [29]. Acridine orange stain and potassium oxalate are used internally in a precision glass hematocrit tube, which contains 55–65 microliters of blood drawn from a punctured finger, ear, or heel. A clear plastic closure is then secured. A precisely designed cylindrical float is inserted, meant to float within the densely packed red blood cells. The tube is centrifuged for five minutes at 12,000 rpm. Distinct bands form as the components of the buffy coat separate based on their densities. The widths of the leukocyte and thrombocyte cell bands, as well as the uppermost region of red blood cells, are ten times larger than usual since the float occupies 90% of the tube's internal lumen. A standard white light microscope, equipped with an epi-illuminated microscope objective and a UV microscope adapter, is used to examine the QBC tube once placed on the tube holder. Fluorescing parasites can then be observed at the interface between white blood cells and red blood cells [30]. The centrifugation process is crucial, as it concentrates the red blood cells in a specific area of the QBC tube, enabling rapid and straightforward detection. Red blood cells infected with Plasmodia are less dense than the normal ones and gather at the top of the erythrocyte column, directly beneath the leukocytes. All adjacent red blood cells are pushed into the 40-micron gap between the float's exterior and the tube's interior. The parasites manifest as bright spots of light among the non-fluorescing red cells because they contain DNA that absorbs the acridine orange stain. By rotating the tube under the microscope, it is possible to detect nearly every parasite present in the 60 microliters of blood. A negative test result can be announced within a minute, while a positive result may be achieved within a few minutes [31].

**Rapid Diagnostic Test (RDT)**

A rapid diagnostic test is a device that uses immunological chromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip to detect malaria antigen in a tiny volume of blood, typically 5–15 µL. It takes 5 to 20 minutes to get the result, which is typically a colored test line. RDTs are straightforward to do, simple to interpret, and do not require electricity or capital expenditure. Compared to the previous assays of the early and mid-1990s, the current RDT test formats (such as in a plastic cassette container or mounted to cardboard) enhance ease of use and safety. The use of RDT has grown in recent years, particularly in developing nations. In June 2007, the U.S. FDA approved one product. Some RDTs differentiate P. falciparum from the three non-falciparum species, but the most widely used RDTs exclusively identify *P. falciparum*. To accommodate the local malaria epidemiology, commercial tests are produced using various combinations of target antigens (http://www.wpro.who.int/sites/rdt/documents). The most often targeted malaria antigen is Histidine-Rich Protein 2 (HRP-2), which is unique to P. falciparum. Certain commercial assays can differentiate between infections caused by P. falciparum (with or without non-falciparum) and those caused by non-*P. falciparum* alone because they include both an HRP-2 assay and an assay for genus-specific aldoses enzyme. The other significant class of targeted antigens are enzymes called parasite lactate dehydrogenase (PLDH). Commercially available monoclonal antibodies against PLDH are used to identify Plasmodium spp. (pan-malaria), *P. falciparum*, and *P. vivax*. The assay specific to *P. vivax* is novel and has not yet been thoroughly tested. Products differ in how test lines are set up and how RDT findings are interpreted. Some products combine a pan-malaria PLDH assay with an HRP-2 assay.

**Serological Techniques**

Malaria parasites can be identified serologically by detecting antibodies specific to certain parasite antigens. Numerous techniques exist, but the two most crucial are the indirect fluorescent antibody test (IFA) and the enzyme-linked immunosorbent assay (ELISA). The Immunochromatographic Test (ICT), which is a quick and highly sensitive serological test, is another method.

**Detection of antimalarial antibodies**

In semi-immune patients in endemic locations, where reinfection is common, antibodies to the asexual blood stages develop a few days after malarial infection, grow in titer over the following few weeks, and last for months or years. After treating a single illness, antibodies in non-immune patients decrease more quickly and become undetectable in three to six months. A subsequent reaction with a rapidly rising antibody titer is induced by re-infection or relapse [24]. Malarial antibodies can be found via enzyme immune assay or immunofluorescence. It can be used to evaluate possible blood donors, conduct epidemiological surveys, and sometimes provide proof of recent infection in non-immune individuals. In the future, evaluating the

response to malaria vaccinations will depend on the identification of protective antibodies.

**Mass spectrometry**

Recently, a new technique for the in vitro detection of the malarial parasite was revealed, with a sensitivity of 10 parasites/µl of blood. It includes a procedure for cleaning entire blood samples, which is followed by time-of-flight mass spectrometry using direct ultraviolet laser desorption. When malaria parasites develop in human red blood cells, they sequester intact ferriprotoporphyrin IX (heme), which produces intense ion signals. The heme's laser desorption mass spectrum is structure-specific, and the parasitemia of the sample is connected with the signal intensities. Numerous samples may be prepared concurrently, and it might only take a second or so to measure each sample. However, current high-tech mass spectrometers cannot operate in isolated rural regions without electricity. This approach may become deployable and practical with future equipment and methodology advancements [29].

**Treatment of malaria**

Treatment for malaria should start as soon as possible because it can be a serious and occasionally fatal illness, especially when caused by *Plasmodium falciparum*. Patients who cannot take oral medications or have severe *P. falciparum* malaria should receive treatment through continuous intravenous infusion. Medications active against the parasitic forms in the blood (the form that causes disease) include chloroquine, atovaquone-proguanil, artemether-lumefantrine, mefloquine, quinine, quinidine, doxycycline (when combined with quinine), clindamycin (when combined with quinine), and artesunate (not licensed in the United States but available through the CDC malaria hotline). Primaquine also prevents relapses and is effective against hypnozoites, the dormant liver forms of the parasite. Pregnant women and those with G6PD (glucose-6-phosphate dehydrogenase) deficiency should avoid primaquine. Patients should not receive primaquine until a screening test has ruled out G6PD deficiency. The treatment a patient receives for malaria is influenced by the species of the parasite causing the infection, the location of the infection, the patient's clinical state, any comorbid conditions, pregnancy, drug allergies, and other medications the patient is taking. Qinghaosu and cinchona alkaloids are natural gifts. Chloroquine, chloroguanide (proguanil), and eventually amodiaquine and pyrimethamine were introduced during World War II [32]. Mefloquine and halofantrine were introduced during the Vietnam War. These medications are currently available to treat malaria. The pharmaceutical industry is not very interested in developing new antimalarial medications, making it difficult to predict the source of the next generation of antimalarial drugs [33]. If treatment resistance in *P. falciparum* continues to grow at its current rate, malaria could become incurable in some regions of Southeast Asia by the beginning of the next millennium. The risks are high, while the returns on investment are modest. The efficacy, safety, accessibility, affordability, and acceptability of antimalarial medications are crucial to the success of early diagnosis and timely treatment, which are the primary technical components of the global strategy to reduce malaria. Effective antimalarial treatment not only decreases malaria mortality and morbidity but also lowers the likelihood of developing drug resistance. Therefore, antimalarial chemotherapy is the cornerstone of malaria control initiatives. However, only three of the 1,223 new drugs registered between 1975 and 1996 were antimalarials, indicating a lack of new medications developed to combat malaria. Thus, a sensible antimalarial treatment program is necessary.

**Toxicity**

Compared to other antimalarial drugs, artemisinin has been associated with less toxic effects. Nausea, vomiting, anorexia, and dizziness are the most often reported hazardous side effects; in many patients, these are most likely caused by acute malaria rather than the medications. Rarely, more severe adverse effects have been reported, such as hemolysis, anemia, neutropenia, and increased liver enzyme levels. With an estimated probability of one reaction per 3000 treatments, there have been two documented occurrences of severe allergic reactions to oral artesunate [34].

**Multidrug resistance**

When the parasite exhibits resistance to more than two active antimalarial drugs with distinct chemical classes and mechanisms of action, it is known as *P. falciparum*. The antifolates (diaminopyrimidine, sulfonamides) and 4-aminoquinolines are often the two classes that are impacted first. The formation of the gametocytes that transmit the resistant genotype is made possible by drug resistance, which causes a delay in or inability to remove asexual parasites from the blood [35].

**Development of resistance**

It is generally known that the malaria parasite frequently undergoes de novo changes, usually one at a time but occasionally several. The resistant mutations persist and spread when there is a high level of infection and insufficient medication levels. A high grade of parasitemia combined with low or insufficient medication levels is necessary for the development of resistance. The majority of resistance cases have come from Southeast Asia. This area has a long history of indiscriminate use of various antimalarial medications and is renowned for having low immunity and low transmission, which result in high parasitemia [5]. Since the majority of malaria infections in these low-transmission areas are symptomatic, a disproportionately higher number of persons undergo treatment, which increases the likelihood that resistant strains will be selected. Additionally, according to one study, *P. falciparum* in Southeast Asia is predisposed to a genetic mutation that results in medication resistance. Africa and other regions with extremely high transmission rates

seem to be less vulnerable to the development of medication resistance. Infections in these regions are contracted from time to time during life, leading to partial immunity (premunition), which in turn regulates the infection, typically at levels lower than those that produce symptoms. Due to asymptomatic infections and frequently unavailable medications in these regions, patients do not get antimalarial medications, which reduces the likelihood that resistance will develop [35]. Immunity reduces the relative transmission advantage of resistance parasites by non-selectively eradicating blood-stage parasites, including the uncommon de novo resistant mutants, and by increasing cure rates even with ineffective medications. Moreover, complicated polyclonal infections in semi-immune individuals may facilitate competition between more-fit sensitive strains and less-fit-resistant strains in the host or mosquito or outbreeding of multigenic resistance mechanisms [13]. Higher gametocyte release from drug pressure promotes the spread of drug-resistant mutants that have eluded treatment. The non-use of primaquine further facilitates such resistance dissemination as a gametocytocidal drug for *P. falciparum*. Therefore, early, suitable therapy is necessary to avoid the generation of gametocytes from the recrudescent-resistant infection [36], in conjunction with primaquine. The administration of medications with protracted elimination phases facilitates the spread of resistant mutant malaria parasites. As a "selective filter," the remaining antimalarial activity that remains after therapy stops infection by susceptible parasites but permits infection by resistant ones. Long after their administration has stopped, medications like piperaquine, mefloquine, and chloroquine, which remain in the blood for months, act as a selective filter [37]. *P. falciparum* has a higher incidence of drug resistance than *P. vivax*, which can be explained by factors that encourage the development of drug resistance being more intense in the former [5].

**Mechanisms of resistance**

The biochemical mechanism of resistance to atovaquone, chloroquine, and antifolates has been well studied. *P. falciparum* isolates that resist chloroquine typically accumulate the drug less effectively than those that are susceptible. Chloroquine-resistant P. falciparum parasites consistently show a polymorphism in the Pfcrt (chloroquine resistance transporter) gene, specifically the single amino acid alteration K76T in the first transmembrane domain. This crucial K76T mutation may alter the selectivity of CRT, allowing chloroquine to exit the food vacuole more effectively (Cooper *et al*., 2005). Although this mutation may not confer resistance by itself, another mutation may occur in the pfmdr1 (multidrug resistance 1) gene, which encodes the transporter responsible for importing solutes into the food vacuole, including the drugs mefloquine, halofantrine, and artemisinin (and potentially chloroquine). In the laboratory, chloroquine resistance in *P. falciparum* can be reversed by combining it with other drugs, including calcium inhibitors, phenothiazines, antidepressants, and antihistamines; however, there is limited clinical data to support this approach, and its applicability to humans remains undetermined [38]. Resistance to mefloquine can also lead to resistance to halofantrine and quinine, and cross-resistance between the 4-aminoquinolines amodiaquine and chloroquine is common [39]. According to Sridaran *et al*., (2010), parasites that are highly resistant to SP possess a combination of triply mutated DHFR and doubly mutated DHPS. Resistance to SP is caused by mutations in the dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) domains, respectively. When atovaquone is used as monotherapy, resistance develops rapidly and is associated with point mutations in the cytochrome b gene [40].

**Identifying drug resistance**

Only by conducting an objective evaluation of therapeutic efficacy can the treatment failure rate be estimated and drug resistance validated. These studies of treatment efficacy are carried out in a controlled setting where the quality and origin of the medications are confirmed, the results of microscopic analyses of blood films are validated, and drug administration is monitored. A human component (immunity), a parasite factor (drug resistance), and individual variation that results in variations in the medicine's availability (pharmacokinetics) all affect the study's outcome. For instance, due to acquired immunity, an adult residing in a high-transmission area may be able to eradicate resistant parasites even if the medication is not completely successful. On the other hand, inadequate absorption may result in treatment failure for a non-immune youngster with severe gastrointestinal issues who has drug-sensitive parasite infections. Additional methods are required to confirm antimalarial drug resistance, even though therapeutic efficacy studies can aid in predicting the risk of drug resistance. It must first be demonstrated that a patient who has just finished therapy has recrudescent parasites. To differentiate between the parasites that generated a new infection and those that are recrudescent, they are genotyped. It is necessary to gather proof that the drug or its metabolites were present in the patient's blood at a sufficient level for at least four parasite cycles. Pharmacokinetic evaluations of blood samples can verify this [35]. Resistance to malaria can be identified and evaluated using a variety of techniques. Drug sensitivity is evaluated in vivo by tracking the clinical and/or parasitological response after therapy. Although drug resistance may be indicated by case reports and passive detection of treatment failure, not all treatment failures may be caused by drug resistance; treatment failure can be caused by a variety of factors, including incorrect dosage, noncompliance with regimen duration, poor drug quality, drug interactions, poor or irregular absorption, and misdiagnosis. First created in 1965, a standardized in vivo test methodology for determining *P. falciparum's* response to chloroquine underwent numerous adjustments until the WHO released the most recent

version in 2003 [38]. Uncomplicated malaria entails a reasonably straightforward, prospective assessment of the clinical and parasitological response to treatment. Additionally, where technically and logistically possible, it allows for the measurement of medication blood levels, an extension of the follow-up time, and the detection of molecular markers to assist in differentiating between reinfection and recrudescence. Early treatment failure is defined as a worsening or less-than-anticipated remission of clinical characteristics and parasite density by the third day following treatment. Late treatment failure is defined as the recurrence or worsening of symptoms after day 4 and the persistence of parasitemia at day 28 (day 14 in areas with high transmission) [38]. The study of antimalarials for uncomplicated malaria should focus on treatment efficacy in children <5 years with clinically evident malaria since younger children frequently show a less favorable therapeutic response to antimalarial medicines than older children and adults. In order to differentiate between treatment failures resulting from pharmacokinetic factors and drug resistance, prospective evaluations should, if feasible, also evaluate blood or plasma levels of the antimalarial [35]. Using in vitro techniques, drug resistance can also be investigated by subjecting finger-prick blood samples on microtitre plates to precisely measured drug concentrations and monitoring for inhibition of schizont development. Other techniques include molecular tests utilizing PCR to detect the presence of mutations encoding drug resistance and animal model studies with in vivo tests carried out in nonhuman animal models [35]. However, the predictive utility of some of these tests is still unknown, and the results of these extra tests should be evaluated cautiously because they do not always correlate well with the findings of therapeutic efficacy studies [35].

**Vaccines**

The most recent recombinant vaccine to be developed is RTS, S. It is made up of the pre-erythrocytic stage of the *P. falciparum* circumsporozoite protein. The CSP antigen triggers the generation of antibodies that can stop hepatocyte invasion and also triggers a biological reaction that allows infected hepatocytes to be destroyed. The low immunogenicity of the CSP vaccination caused issues throughout the studies. By combining the protein with a hepatitis B surface antigen, RTS, S sought to circumvent them and produce a more effective and immunogenic vaccine. The vaccine provided protective immunity to seven out of eight volunteers when they were challenged with *P. falciparum* in studies using an oil-in-water emulsion with the adjuvants of monophosphoryl A and QS21 (SBAS2).

**Prevention and control measures for malaria**

Malaria, a major cause of mortality in endemic nations, has both direct and indirect impacts on hematological parameters. However, certain hematological indicators in populations residing in malaria-prone areas have not been consistently reported as standards for gauging malaria burden [41]. Currently, chemoprophylaxis and prevention from mosquito bites are the two complementary strategies used to prevent malaria. Although there are a number of malaria vaccines being developed, none are yet accessible [42].

**Chemoprophylaxis**

To decide which medication is advised for chemoprophylaxis, travelers to malaria-endemic countries—which are divided into three or four categories—are the only ones eligible for malaria chemoprophylaxis. The length of possible vector exposure, the pattern of parasite resistance, the degree and seasonality of transmission, age, and pregnancy all influence the medication selection. Depending on the degree of endemicity and the seasonality of transmission, chemoprophylaxis may also be advised for pregnant women and small children with autochthonous disease in endemic countries.

**Mutation at Position 86 of the pfmdr1 Gene**

Current Results in Sudan: All samples in the current study exhibited the wild type at position 86 with no mutations, suggesting stability at this position in the Sudanese samples studied. The current study identified six mutations at position 184, with Y184F being the most common at 53.3%. This mutation is believed to increase the parasite's sensitivity to drugs like lumefantrine and artemisinin. Studies Inside Sudan Previous studies in Sudan showed moderate prevalence of the Y184F mutation in central and northern regions, with slight geographic variation. These studies also indicated an association with the effectiveness of artemisinin-based combination therapies (ACTs), which aligns with the current study's results. In regions such as Southeast Asia and South America, the Y184F mutation is more common and linked to increased sensitivity to artemisinin treatments, especially in areas with extensive use of these drugs. The higher mutation rate in Sudan may suggest a similar effect, potentially related to variations in drug policies or the parasite's genetic patterns. The study revealed geographical variation in the mutation distribution, with higher prevalence in the DMZ and SNG regions. However, no significant statistical correlation was found between geographic regions and mutation patterns. Other studies in Sudan confirmed that regions with high malaria transmission rates (such as the eastern endemic areas) often show resistance mutations, supporting the current study's findings. Studies in countries like Uganda and Tanzania show mutations are strongly associated with high transmission areas, indicating a strong link between geography and parasite pressure. However, the geographic variation in Sudan is less pronounced than in these regions.

## CONCLUSIONS AND RECOMMENDATION

In Sudan, artemisinin continues to be a safe and effective first-line treatment for uncomplicated falciparum malaria. However, the presence of certain

mutations can result in treatment failures. Evidence indicates that this gene has disseminated across Sudan and may persist, as the mutation allele associated with malaria treatment resistance has been identified in various regions. These drug-resistant genes could hinder the development and execution of malaria control strategies in different areas of Sudan. While the prevalence of malaria parasites varies by region, there is no correlation with the Pfmdr-1 gene polymorphism or gender. The nation's insufficient use of anti-malarial drugs heightens the risk of drug failure from sub-therapeutic doses, making it crucial to monitor artemisinin's effectiveness. Additionally, tracking molecular markers linked to artemisinin resistance is essential for evaluating in vivo efficacy. The recommended treatment for uncomplicated falciparum malaria is the second-line therapy, currently under observation in Sudan. However, to pinpoint any pharmaceutical failures, further studies are urgently required in diverse regions of Sudan. This includes employing sequencing to identify all variants of drug-resistance genes. Your involvement in this study is critical. Comprehensive research and the development of effective strategies are essential to thwart the spread of the parasite.

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