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# *Plasmodium falciparum* Protein Exported in Erythrocyte and Mechanism Resistance to Malaria

Neyder Contreras-Puentes

## Abstract

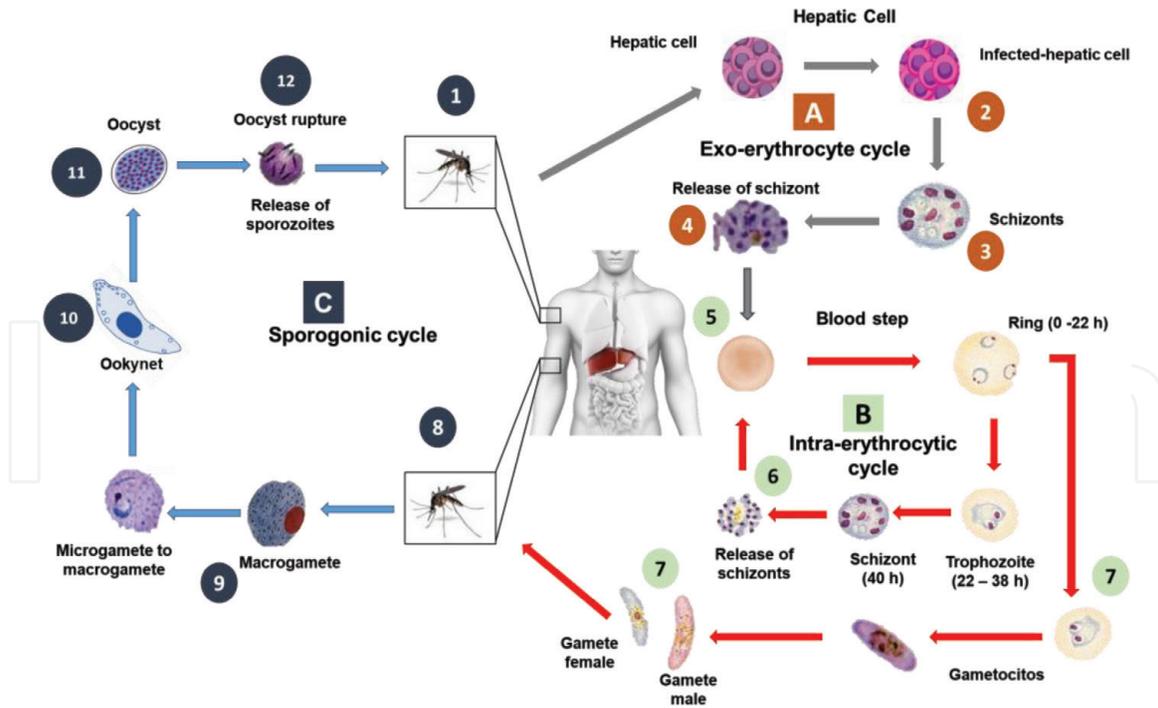
Malaria is a tropical disease of parasitic origin transmitted by the Anopheles mosquito, caused by the protozoan of the genus *Plasmodium*. Around miles of people worldwide affected by disease, have been related the endemic development of genetic alterations, called erythrocyte polymorphisms. These erythrocyte polymorphisms have become tools for resistance against malaria, where they have had an impact on the appearance of hemoglobinopathies, enzymatic alterations in erythrocytes, and modifications in the structure of erythrocytes related to membrane proteins. These sections address a detailed approach to the resistance mechanisms involved against the development of *P. falciparum* and develop a complete development of the principles of molecular principles that attempt to explain the functioning of these biochemical mechanisms and the development of the parasite.

**Keywords:** malaria, *Plasmodium falciparum*, erythrocyte, polymorphism, protein, hemoglobinopathies

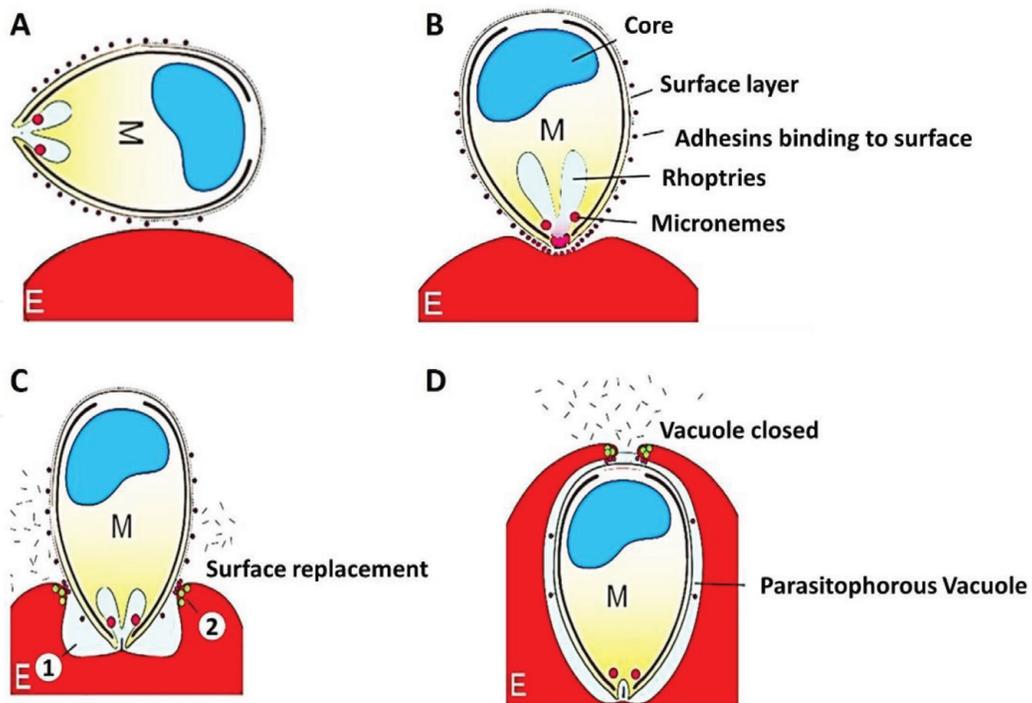
## 1. Introduction

Malaria is one of the world's most severe public health problems. It leads to high rates of morbidity and mortality in many underdeveloped countries, where children and pregnant women are the most affected groups. According to the World Malaria Report by the World Health Organization (WHO), 3.5 billion people from 106 countries live in areas where they are in risk of transmission, representing half of the world's population [1]. On the other hand, malaria caused an estimated 200 million clinical episodes and 445,000 deaths, 90% of these deaths in Africa [2, 3]. Malaria is caused by parasites of the *Plasmodium* genus, which are intracellular eukaryotic organisms, with a complex life cycle. They commute between an invertebrate transmitter vector, where the sexual stages develop, and a vertebrate host, where the asexual stages take place. *P. falciparum* is responsible for the severe forms of malaria and the majority of annual deaths [4, 5].

Human malaria clinical signs and symptoms are a direct consequence of the parasite's life cycle. Humans are infected with *P. falciparum* sporozoites, through the female Anopheles mosquito's bite. Each sporozoite reaches the liver through blood or lymphatic circulation and multiplies forming a liver schizont, which differentiates into thousands of merozoites that are released into the bloodstream, after the schizont ruptures. Once released into the systemic circulation, the merozoites invade



**Figure 1.** Life cycle of *Plasmodium* spp. A. Exoerythrocytic cycle (1). *Anopheles mosquito* inoculates the sporozoites with subsequent invasion in liver cells (2); generation of first pre-erythrocytic schizogony (3). B. Erythrocytic cycle. The rupture of the schizont (4) releases the merozoites into the bloodstream where they invade red blood cells (5) forming a trophozoite that ripens into schizont, whose rupture releases merozoites back into the torrent (6). Some trophozoites can mature into gametocytes (7) that are ingested by the mosquito (8). C. Sporogonic cycle. The gametocytes mature to macrogametes and flagellated microgametes (9) that, after fertilization, produce an ookinete (10), which migrates from the mosquito to generate oocyst (11) that will release thousands of sporozoites (12). Adapted from [http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Malaria\\_il.htm](http://www.dpd.cdc.gov/dpdx/HTML/ImageLibrary/Malaria_il.htm).



**Figure 2.** Merozoite invasion process in human erythrocytes. Description of invasion and internalization of the *P. falciparum* parasite in the host cell. (1) The nascent parasitophorous vacuole. (2) Contact closed. (A) Initial contact of merozoite to erythrocyte. (B) The adhesion of the merozoite to the erythrocyte is observed, through the specific recognition and interaction of antigens and antibodies, as well as the functional and structural role of the micronemes and rhoptries. (C) Process of invasion and development of the parasitophorous vacuole. (D) The internalization of the merozoite in the new host cell and the complete formation of the parasitophorous vacuole are detailed. Taken and adapted from Zuccala and Baum [30].

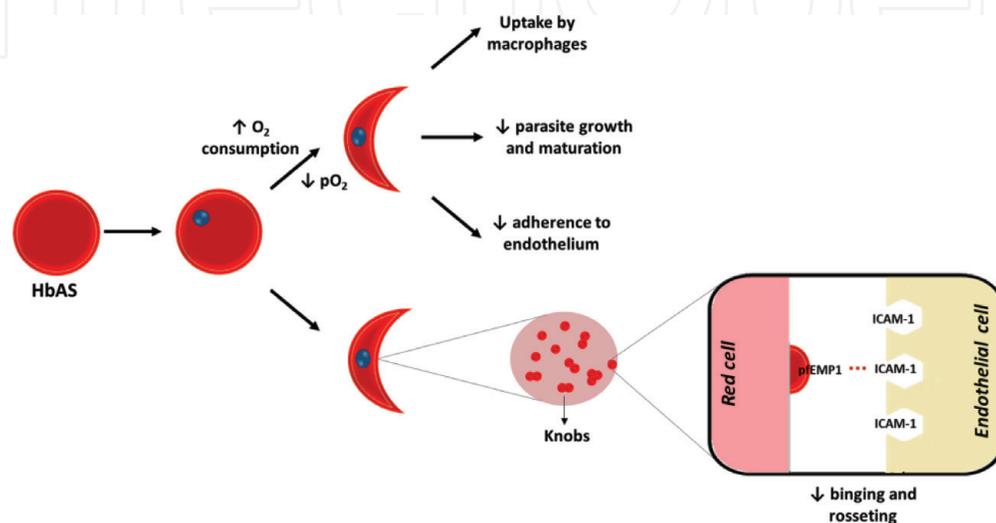
the red blood cells and initiate the intra-erythrocyte stage, which lasts approximately 48 hours. Immediately after the invasion, the growth and development staging begins first as rings (0–24 h), then as trophozoites (24–40 h), and finally as schizonts (40–48 h); the cycle ends with the host cell destruction and the release of new merozoites from circulating erythrocytes, then initiating another cycle [6] (**Figure 1**).

During the development and growth stages, the parasite causes successive changes in the architecture of the infected erythrocyte (remodeling), which are fundamental for its vital functions. These changes are the acquisition of extracellular environment nutrients, the attribution of cytoadhesive properties that contribute to spleen-clearance evasion, the generation of changes in the host membrane cytoskeleton that are necessary for efficient parasite progeny release, and the formation of new organelles, such as the Maurer's clefts, tubulovesicular network, and the parasitophorous vacuole membrane (PVM) (**Figure 2**) [7, 8]. When the parasite enters the erythrocyte, it locates inside a parasitophorous vacuole (PV), which isolates it from the host cell cytoplasm, through the PVM. From then on, pathogen survival will depend on the efficient traffic of the molecules through the PVM and the plasma membrane [4, 9].

## 2. Human erythrocyte: properties of the human erythrocyte membrane

The erythrocyte is a cell of approximately 8  $\mu\text{m}$  in diameter, highly specialized in  $\text{O}_2$  and  $\text{CO}_2$  transportation, without a nucleus and other organelles, useful for protein synthesis. It has the ability to transit the bloodstream over a 120-days lifetime. In addition, it has a remarkable capacity for deformability that allows its movement through the capillary microcirculation and splenic endothelial clefts in approximately 1  $\mu\text{m}$  diameter [10]. The erythrocyte elastic properties are due to the cytoskeleton membrane, which is formed by an array of regular hexagonal proteins which makes up a two-dimensional mesh on the cell's cytoplasmic surface. These structural proteins interact with membrane lipids to maintain fluidity and subdivide them into three protein types: cytoskeleton, integral, and anchor [11].

The membrane cytoskeleton proteins underlie just under the lipid bilayer and associate with other proteins, forming a dynamic protein network, responsible for maintaining the integrity of the erythrocyte, as it passes through narrow blood capillaries. Spectrin, actin, adducin, dematin, band 4.1, tropomyosin, and tropomodulin are within this group. Integral proteins are characterized for being embedded in the lipid bilayer and presenting intra and extracellular domains, such



**Figure 3.**  
Mechanisms of HbAS-related protection against *P. falciparum*. Adapted of Bunn [81].

as band 3 and glycophorin A and C. Finally, anchoring proteins have the function of connecting the cytoskeleton proteins with integral proteins, such as ankyrins and band 4.2 proteins [12, 13].

### 3. Erythrocyte and merozoite membrane proteins: participate in *P. falciparum* invasion process

The remodeling of the structures of the human erythrocytes for parasite of the malaria is generated between the process of invasion of merozoites. During the process, the parasite induces a transitory alteration of the structure of the membrane of the host cell, binding sites of the surface cellular [14]. More than 50 *P. falciparum* proteins have been identified which induce the process of invasion; however, some functional classes of elements such as merozoite surface protein (MSPs) have been described, which have demonstrated a structural complex around the envelope of the merozoites, related to PfEBAs (*P. falciparum* erythrocyte binding antigens) and PfRHs (*P. falciparum* reticulocytes binding protein), which are able of save organelles as micronemes and rhoptries [15–17].

In general, the erythrocyte membrane changes are set in motion with the merozoite invasion process. It has been described that the initial interaction between the merozoite and erythrocyte is probably a random collision, depending on the function of actin-myosin binding and specific molecular interactions between merozoite ligands and erythrocyte membrane receptors, which mediate cellular recognition and invasion of red blood cells [18]. This invasive process is carried out in four steps. The first step, called the initial contact with merozoite, takes place mainly by the interaction of proteins that are uniformly distributed on the surface of the merozoite, called glycosyl-phosphatidyl-inositol protein (GPI), with erythrocyte surface ligands, such as merozoite surface protein 1 (MSP1), whose receptor is band 3 protein in the erythrocyte membrane [19, 20]. The second step is called reorientation, which is produced for vertical arrangement of apical secretory organelles, such as rhoptries and micronemes. This step is mediated by a protein called apical membrane Antigen-1 (AMA1), which seems to establish the apical interaction of the adhesins with the erythrocyte; it is the border point between the weak union that occurs in the initial contact with MSP1 and irreversible bonds that occur between microneme proteins and erythrocyte membrane proteins [21, 22]. The third step is the tight-binding formation between various adhesins produced at the apical end of the parasite and its membrane receptors in the red blood cell, where the Duffy binding-like proteins (DBL) and reticulocyte binding proteins (RBP) bind. For example, surface DBL proteins of merozoite EBA 175 and EBA 140 (erythrocyte binding antigen 175 and 140) bind to erythrocyte membrane sialoglycoproteins, such as glycophorin A and C [23, 24]. On the other hand, while PfRh proteins bind to complement receptor 1 (CR1), signalization established by sensitive chymotrypsin receptor pathway and resistant to neuraminidase takes place [23, 25]. Once the parasite and erythrocyte tight junction is established, intake is mediated by the actin-myosin motor activation on the merozoite surface. This coincides with lipid and protein secretion, such as organelle-released proteases called rhoptries, micronemes, and mononemes. These proteases are associated to perform integral membrane proteins cleavage, such as band 3 and rupture of the membrane cytoskeletal proteins [26, 27].

During the invasion, proteins from the rhoptries and dense granules are secreted into the parasitophorous vacuole, and once it has developed to the ring phase, these proteins are exported to the cytoplasm of the infected erythrocyte to trigger the succession of effects of remodeling at the level of the host cell. It has been established that *P. falciparum* is capable of associating with Knobs, which are related

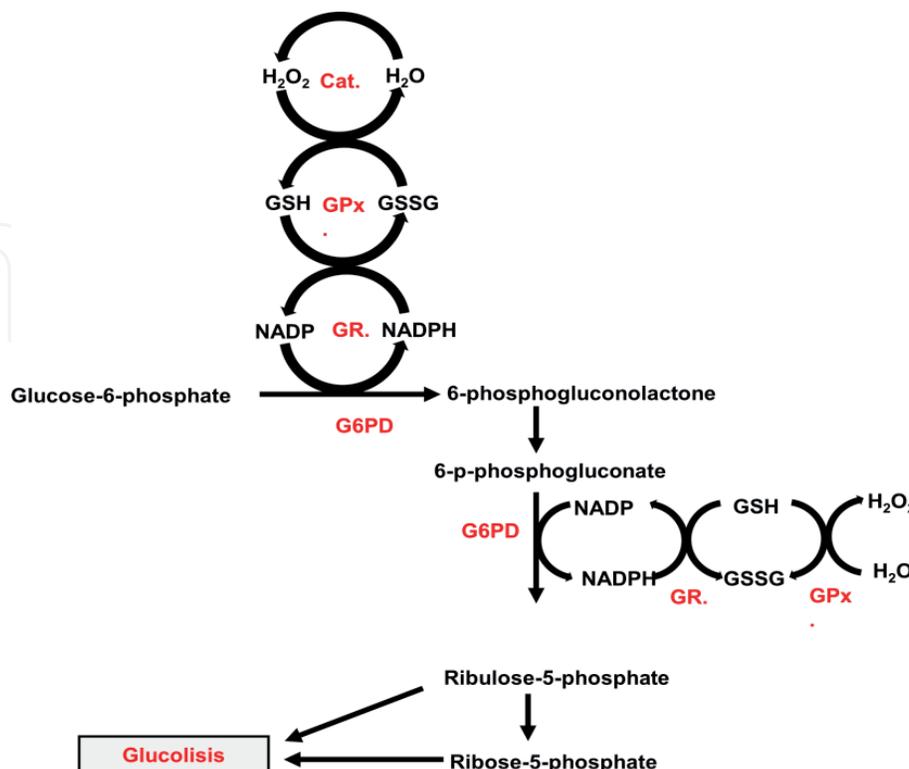
to Knobs-proteins rich in histidines (KHARP). This type of formations allows the presentation of cytoadherence proteins exported by the parasite, which are coupled to the membrane, as is the case in particular of *P. falciparum* of erythrocyte membrane protein 1 (PfEMP1) [27].

Subsequently, the parasite invaginates the erythrocyte through a protein-free zone and initiates the formation of parasitophorous vacuole, which continues with a motility mechanism to enter the host cell. Rhoptries and dense granules secrete proteins during the invasion early ring phase, which are trafficked to different structures, such as parasitophorous vacuoles, cytosol, and erythrocyte membrane, triggering a series of events that modify the host cell [28].

#### 4. *P. falciparum* export proteins modify the erythrocyte membrane.

Once inside the erythrocyte, *P. falciparum* is subjected to a trophic phase, followed by a replicative phase. The parasite modifies the host cell during the intra-erythrocytic period, conditioning it as its new habitat. It induces the formation of new permeability pathways, allowing it to provide itself with essential nutrients, dispose of waste products, modify the electrolytic composition, and decrease the colloid osmotic pressure of the erythrocyte, in order to survive in this new environment [29].

The infected erythrocyte enlarges in size, developing the formation of parasitophorous vacuole (PV), parasitophorous vacuole membrane (PVM), new membranous structures, such as the Maurer's clefts (MC), tubulovesicular networks (TVN), and erythrocyte surface protrusion appearance called Knobs. Moreover, new type of channels in the PVM and alterations of the erythrocytic membrane channels are formed, in which virulence proteins are trafficked [7, 29, 30]. In addition to MC and TVM, other structures have been described, which are involved in export protein trafficking, such as electron-dense vesicles (EDV), vesicle-like structures (VLS), J points or J-Dots, named for J-domain proteins [31–34].



**Figure 4.** Glucose-6-phosphate dehydrogenase (G6PD) pathway. G6PD, glucose-6-phosphate dehydrogenase, GPX, glutathione peroxidase; GR, glutathione reductase, 6PG, 6-phosphogluconate dehydrogenase; GSH, glutathione reduced; GSSG; glutathione oxidized. Adapted from Cappellini and Fiorelli [92].

Name of identification	Protein	Location	Molecular weight (kDa)	Putative function	References
KAHRP	Knob-associated histidine-rich protein	Erythrocyte cytoskeleton	85–105	Essential for the formation of Knobs; joins the erythrocyte spectrin, actin, and cytoplasmic tail of PfEMP-1	[40, 41]
MESA/PfEMP2	Mature parasite-infected erythrocyte surface antigen	Erythrocyte cytoskeleton	168	It binds to the protein 4.1R. You can interrupt the interaction p55–4.1R	[41–43]
RESA/Pf155	Ring-infected erythrocyte surface antigen	Erythrocyte cytoskeleton	127	Joins the spectrin. Suppresses the increase of heat-induced membrane. It can stabilize the erythrocyte membrane. Could prevent the invasion of erythrocytes parasitized	[44–46]
Antigen 332 (Pf332)	<i>P. falciparum</i> antigen 332	Erythrocyte cytoskeleton and Maurer's clefts	700	Binds with the protein actin and provides deformability of erythrocytes	[47]
GBP130	Glycophorin binding protein 130	Erythrocyte cytoplasm and membrane of parasitophorous vacuole	105	Decrease of rigidity	[48]
PfEMP3	<i>P. falciparum</i> erythrocyte membrane protein 3	Erythrocyte cytoskeleton and Maurer's clefts	274–315	Joins the spectrin. Interrupts the interaction of the actin-spectrin-4.1R protein complex. Involved in the trafficking of PfEMP1.	[41, 49]
PfEMP1	<i>P. falciparum</i> erythrocyte membrane protein 1	Erythrocyte membrane and Maurer's clefts	200–250	Cytoadherence ligand, antigenic variation, and interacts with KARHP	[50]
RIFIN	Repetitive interspersed family	Maurer's Clefts and erythrocyte surface	—	Possibly antigenic variability	[38]
STEVAR	Subtelomeric variable open reading frame	Maurer's Clefts and erythrocyte surface	—	Possibly antigenic variability	[51]
SURFIN 4.2	Surface-associated interspersed gene protein 4.2	Maurer's Clefts and erythrocyte surface	—	Possibly antigenic variability	[52]

Name of identification	Protein	Location	Molecular weight (kDa)	Putative function	References
MAHRP1	Membrane-associated histidine-rich protein 1	Erythrocyte membrane	28.9	Generating the Maurer's clefts or in protecting proteins within these structures	[53]
REX-1	Ring-exported protein 1	Transmembrane	83	ND	[54]
ND, nondeterminate.					

**Table 1.**  
 Proteins from the export of *P. falciparum* that modify post-invasion erythrocyte [6, 9].

Another host cell modification refers to *P. falciparum* infected erythrocyte cytoadherence to endothelial cells, resulting in a sequestration of mature parasites in capillaries and microvasculature [35]. The sequestration probably leads to microcirculation alterations and metabolic dysfunctions, which could be responsible for severe malaria manifestations [36]. The cytoadherence to endothelial cells confers at least two advantages for the parasite: (1) a more suitable microaerophilic environment for parasite metabolism and (2) evasion to splenic circulation, where infected erythrocytes would be destroyed [36–39]. *P. falciparum* exports its proteins to the erythrocyte cytoplasm, where it binds to cytoskeletal components and alters the natural interactions of the membrane structural proteins, in order to achieve these major changes in the erythrocyte structure. Export proteins are encoded by 8% of *P. falciparum* parasite genome. It corresponds to host cell exported proteins, both in asexual and gametophytic phases. **Table 1** lists the main *P. falciparum* export proteins, which participate in the remodeling process and present PEXEL motifs. However, non-PEXEL proteins such as PfEMP1, SURFIN, and Pf332 are also shown in **Table 1**, due to their importance in the infected erythrocyte remodeling process, but only PfEMP1.

## 5. Mechanism of resistance to malaria and effect of *P. falciparum* in erythrocyte

Parasites of the genus *Plasmodium* have co-evolved over 200 million years with the human species [55]. In this way, the increase of migrations in multiple regions and the establishment of settlements in certain areas have influenced the increase in endemicity produced by the successive exposure of the etiological agent of the disease; this high effect of selective pressure of the parasite has co-influenced the development of genetic variations linked to endemic populations, from which they have emerged over time polymorphic variants in erythrocytes in order to respond to the most severe symptoms of the disease, hindering the survival of the parasite or preventing the development of its entire life cycle. Many of these variations may be due to changes in structural proteins of the erythrocyte, alterations in hemoglobin (thalassemia's and sickle cell anemia), or an incidence in the quantitative and functional level of enzyme activity involved in oxidative processes such as G6PD or pyruvate kinase [56, 57].

Currently, a global distribution of erythrocyte polymorphisms has been described, such as hemoglobinopathies (thalassemia's, HbS, HbC, and HbE) and enzymatic alterations such as glucose-6-phosphate dehydrogenase deficiency (G6PD), which have their origin in response to the selective pressure exerted by

malaria parasites on humans during the last 70,000 years [58]. Therefore, hemoglobinopathies and erythroenzymopathies have been attributed to different mechanisms that provide protection against severe manifestations of malaria; some of the relevant mechanisms are associated with reduced erythrocyte invasion, decreased intraerythrocytic growth, increased phagocytosis in infected erythrocytes, and increased immune response against parasitized erythrocytes [59]. Therefore, this type of erythrocyte polymorphisms can be related to resistance to malaria through immune mechanisms that can be a major health problem, due to the high frequency of carriers in endemic areas of malaria, mainly in the African continent where this It seriously affects the normal development of populations. Therefore, this type of genetic variants was originally characteristic of the tropics and subtropics; nowadays, there is a high dispersion in the whole world, product of the continuous migrations that induce an increased effect of the prevalence values of these diseases [60].

## **5.1 Hemoglobinopathies**

Hemoglobinopathies are a group of genetic alterations that involve a change in some of the subunits of hemoglobin and present an autosomal recessive inheritance pattern [61, 62]. These are divided into structural hemoglobinopathies, produced by the simple substitution of amino acids in the  $\alpha$  and  $\beta$  chains of hemoglobin and thalassemic syndromes, which are manifested by the total or partial decrease in the synthesis of a globin chain [63]. The frequency of these polymorphisms in the world population and their geographical distribution are highly variable. In the case of hemoglobinopathies, it is estimated that every year more than 300,000 children with severe forms of these diseases are born worldwide, most of them in countries of low and medium income [64, 65]. Approximately 5% of the world population carries a sickle cell or thalassemia gene, and in some regions, the percentage of carriers can reach 25%. Approximately 60–70% of all births of children with some serious alteration of hemoglobin (Hb) occurs in Africa, being the sub-Saharan region the most affected [66, 67].

### *5.1.1 Hemoglobin S*

Hemoglobin S (HbS) is associated with a mutation in the  $\beta$ -globin gene where there is a change of thymine by adenine, thus coding a valine instead of glutamic acid (Glu6Val,  $\beta$ S). This mutation produces a hydrophobic modification in the deoxygenation of the Hb tetramer, which results in the union between the beta-1 and beta-2 chains of the two hemoglobin molecules (Hb). This union produces a polymer nucleus, which grows and invades erythrocytes, affecting architecture and flexibility and influencing cellular dehydration, with physical and oxidative cellular stress [68]. HbS is a hereditary trait that follows an autosomal recessive pattern, and therefore, it can present in a homozygous (HbSS) or heterozygous (HbAS) form. The HbSS form causes sickle cell anemia (SCA), while the heterozygote is considered a carrier of the trait [69].

It is estimated that around of 300 million people in the world, are diagnosed with sickle cell trait (SCT) a greater presence in Africa and the Mediterranean region, where the endemic areas of malaria are related to the occurrence of these hemoglobinopathies. In the United States, the prevalence of sickle cell traits is estimated at 8% for African-Americans and 0.05% for white Americans, suggesting an approximate incidence of 7.9 per 100,000 births. [70, 71].

At the metabolic level, it has been described that an increase in the production of ROS in the erythrocytes of individuals carrying the sickle trait shows a behavior similar to senescent erythrocytes. This phenomenon describes that aging causes erythrocyte cytosolic changes that affect antioxidant functioning, which can lead to the

generation of a redox imbalance and induce the hemolysis and toxic accumulation of heme and Hb in the plasma [72, 73]. This oxidative imbalance tends to be even greater in the erythrocytes of carrier individuals, a process that increases in cytosolic and membrane transformations due to the decrease in its half-life. Hence, the infection of HbAS erythrocytes with malaria parasites causes an increase in the redox imbalance associated with the metabolic activity of the pathogens. However, the molecular effects of this imbalance are not fully established and therefore it is necessary to continue with their study to establish their role in the parasitic-host relationship.

Equally, have been suggested mechanisms which the sickle cell protects against malaria as shown by Pasvol et al., where an inhibition of growth of the parasite due to the polymerization of HbS and effect related with low oxygen levels is presented [74, 75]. Recently, Archer et al. demonstrated that infected erythrocytes HbAS showed a decrease in oxygen levels affecting the intracellular growth. These investigations have evidenced that growth inhibition produced by HbS-polymerized increments the cytoadherence, a condition favorable for inducing a reduction in the development of parasites [76].

Other mechanisms related have evidenced morphologic modifications in erythrocyte due to aberrant expression of PfEMP1 able of affect the binding of infected RBCs to host cells, and induce the diminution of virulence through the reduction of rosette formation and decreased cytoadherence [77]. Also, it has been described that the generation of antibodies against band-3 protein may be associated with formation of aggregated band-3 with impact in new sites for endothelium adhesion on erythrocyte with such polymorphisms and finally able cause conformational changes in band-3. Alike, it has been demonstrated that the parasite remodels the interaction of actin-cytoskeleton binding to enable the export of parasite-derived proteins to knobs on the parasitized RBC surface [58] **Figure 3**.

Thus, the mechanism established that during the invasive step in sickle cell, all are affected to a phenomenon of oxidative stress. This increase in ROS induces phagocytosis phenomenon related with hemoglobin denaturation, formation and hemichrome binding, aggregation protein as band 3 protein, development of antibody and its deposition, and binding of complement C3c fragments [78].

In this way, the increase of phagocytosis processes in HbAS erythrocytes infected with *P. falciparum* is remarkably advantageous for the host, in which a succession of associated mechanisms is triggered such as growth reduction and population density of parasites, young forms of the parasite are rapidly eliminated by the immune response, and it has been observed that mature forms (trophozoites and schizonts) adhere to the endothelium in smaller proportion in important organs (lungs, kidneys, brain, bone marrow, and placenta), which has led to a decrease in the severe symptoms of the disease (cerebral malaria, placental malaria, and respiratory disorders) [79, 80].

On the other hand, some molecular mechanisms have been established which have included the concept of microRNA (miRNAs). The development in cultures have founded the action of two miRNAs, miR-451 and let-7i, regulating of growth of parasites. Likewise, the incidence of miR-451 and let-7i have induced reduction of parasitemia and a notable effect in the incorporation of hypoxanthine producing changes in characteristic of erythrocyte and defects during invasion of parasite, which have been associated with high specificity of sequences of miRNAs with anti-parasite function.

### 5.1.2 $\alpha$ - and $\beta$ -thalassemia

During much time, the association between  $\alpha$ -thalassemia and malarial protection mechanism has been studied, reporting the presence of the  $\alpha$  + variety in the

studied population [82]. The  $\alpha$ -thalassemia is able to induce hemolytic state and be associated with a reduction in erythrocyte survival, with an increased erythrocyte in circulating young erythrocytes [83]. The  $\alpha$ -thalassemia is very common in malaria-endemic regions; it is considered to confer protection against clinical manifestations of the disease induced by *P. falciparum*. *In vitro* studies have evidenced that in  $\alpha$ -thalassemic erythrocytes infected with *Plasmodium*, high levels of antibodies develop from their surface. Additionally, activation mechanisms in opsonized erythrocytes, complement-induced lysis and inhibition of sequestration of infected erythrocytes have been associated, which result as anti-malarial mechanisms that might be promoted by such antibodies [84, 85].

In other studies, the roles of microcytosis have been associated with the protection from *P. falciparum*-related hemoglobin decrease; in patients, a reduction of infection for part of parasite and most notary in homozygous  $\alpha$ -thalassemic individuals have been evidenced, where a decline of hemoglobin levels, is observed and likewise, microcytosis is related with oxidative stress induced in altered erythrocytes with the presence of thalassemia and iron-deficiency. Finally, could be linked a development of process as low resetting in infected microcytic RBCs [86]. Likewise,  $\alpha$ -thalassemia protects against severe malaria by attenuating the effect of parasite virulence and decreasing the amount of Hb loss during increased parasitemia. The  $\alpha$ -thalassemia erythrocytes parasitized may be more susceptible to phagocytosis in vitro culture and unavailable than normal red cells in the formation of rosettes [87, 88]. Alike, has been related the complement receptor 1 (CR1), which is reduced on  $\alpha$ -thalassemic erythrocytes infected, the diminution of CR1 expression in this type of cells are associated with a possible mechanism for reduction resetting [89]. Following, with less able to adhere to endothelial cells. Of this mode, studies have suggested that altered cells maintain that membrane band 3 may be a target for enhanced antibody binding to parasitized  $\alpha$ -thalassemic cells [90, 91].

## 6. Erythroenzymopathies

### 6.1 Glucose-6-phosphate dehydrogenase deficiency (G6PD)

Worldwide, one of the most frequent polymorphic disorders at the level of erythrocytes is the deficiency of glucose-6-phosphate dehydrogenase (G6PD), a condition that is triggered by the decrease in the activity of glucose-6-phosphate dehydrogenase [92]. This disorder linked to genetics is located in the terminal region of the long arm of chromosome X (Xq28) and characterized by establishing the condition of deficiency or normal in men; and in the case of women, it is established that they can be heterozygous, homozygous, or normal [92, 93]. The heterozygous women have a copy of the gene that synthesizes the normal G6PD and another copy that produces the variant of the enzyme.

The active enzyme consists of identical subunits that form dimers and tetramers, which contain a nicotinamide-adenine dinucleotide phosphate (NADP) binding site [94, 95]. NADP binds to the enzyme, as a structural component and as a substrate for the reaction. As shown in **Figure 4**, G6PD catalyzes the entry of glucose-6-phosphate (G6P) into the pentose phosphate pathway, specifically that of hexose monophosphate, a reaction that produces glucose-6 oxidation, phosphate to 6-phosphogluconolactone, reducing NADP to NADPH [96].

In erythrocytes, it is the only source of NADPH, being essential to protect cells against physiologically high levels of oxidative damage, enzymatic mechanisms

associated with increases in reduced glutathione (GSH) [92]. Where glutaredoxin intervenes and by means of which GSH protects the sulfhydryl groups of the hemoglobin and the erythrocyte membrane, but in the presence of oxidizing agents, in the form of free radicals or peroxides, the level of GSH decreases, although it can be restored by the action of glutathione reductase which does have an adequate NADPH supplement [75].

Wide mechanisms have been described for the study of role of G6PD-deficiency as elements protective during infection with *P. falciparum*. The distribution in the world with respect to malaria is similar to the mutated alleles G6PD; these observations have evidenced that first studies evaluated the connection between G6PD deficiency and malaria, with contradictory results. However, the allelic heterogeneity of G6PD deficiency may be related with susceptibility of *P. falciparum* when infected erythrocytes are present under this condition. Thereby, studies established by Ruwende and col. have demonstrated that G6PD A- alleles are associated with a reduction in the risk of severe malaria caused by *P. falciparum*, protection that are confer principally in heterozygote individuals [97]. Likewise, experimental investigations have evidenced a diminution in the growth of parasitized-erythrocytes with G6PD A and A- in Mediterranean population when contrast with normal subject. Thus, this has indicated the incidence of mechanism of initial phagocytosis, where infected RBC of G6PD-deficients is induced to phagocytosis by macrophages in anterior stages of the development of parasite, an aspect that is related with protective mechanism against malaria [98, 99]. Equally, a direct relationship of activation of process as phagocytosis in ring stages of parasite in erythrocytes infected with this condition has been considered [99]. This mechanism is associated with an increased binding of autologous IgG and complements C3 fragments when were compared with infected-RBC normal individuals [100]. Finally, have been associated a succession of phenomena's as the oxidation under increase of ROS into the erythrocytes and formation aggregated of band-3 protein [101].

## 6.2 Pyruvate kinase deficiency (PK)

Pyruvate kinase (PK) is an enzyme engaged in the conversion of phosphoenolpyruvate (PEP) to pyruvate. The catalysis of PK is an important element for formation of ATP in the glycolytic route [102]. PK plays a fundamental role in erythrocyte due to which cells depend on the production of ATP by glycolysis for the metabolic development and functionality of the cells [103]. The PK activity generally is increased in erythrocytes in the infection process. Likewise, have been associated to recognizing and the generation of the target of drug with *P. falciparum* infection [104]. PK deficiency is enzymatic alteration of the glycolytic route inducing non-spherocytic hemolytic anemia. The cause frequently linked is due to punctual mutations (1529A and 1456 T). PK deficiency presents worldwide distribution and is commonly prevalent in Caucasian populations [105].

It has been shown that PK deficiency is related to protection against infection in mice with *Plasmodium chabaudi* parasites, suggesting a similar effect of PK deficiency in humans. These effects have shown that PK-deficient human erythrocytes have induced diminution of malaria infection [106]. Other reports have indicated that possibly a protective effect against *P. falciparum* infection is generated, with alterations associated to replication on infected erythrocytes, where an invasive defect of erythrocytes in subjects bearing the homozygous mutation and to a preferential macrophage clearance of ring-infected erythrocytes is evidenced both in homozygous and heterozygous individuals [107].

Some phenomena have been associated with the deficiency of pyruvate kinase and infected erythrocytes, such as those established by the pleiotropic effect of the enzyme deficiency in the invasion of the parasite, which favors a substantial reduction of the growth of these and in the same way observes the activation of processes such as phagocytosis of infected erythrocytes in the ring stage that can provide protection against malaria, either by causing a reduction in the parasite burden or by reducing the number of erythrocytes infected with parasites in the trophozoites stages and schizonts that are available to join microvascular beds of vital organs [108].

These result in a reduced level of invasion of *P. falciparum* and erythrocytes of subjects with homozygous mutations. We also indicated that the possible biochemical differences in the intracellular medium, including the accumulation of glycolytic metabolic intermediates, did not cause a difference in the growth of parasites in erythrocytes between homozygotes and heterozygotes [109, 110]. To know even more the hypotheses of the reduction of the invasion observed in the erythrocytes of the subjects with homozygous mutations, it is also due to the capacity of the parasite, including the altered development of merozoites, the invasion of erythrocytes by merozoites was examined. It has been observed that the erythrocyte-tale merozoites had normal levels of invasion and replication in the erythrocytes of the control subjects [110].

We examined the phagocytic uptake of infected erythrocytes with *P. falciparum* (ring phase and mature phase) of case and control matter. Phagocytosis of infected erythrocytes in the ring stage of patients with homozygous mutations was higher than phagocytosis of uninfected erythrocytes. Also, an increased clearance has been observed by macrophages of erythrocytes infected in the ring stage of the parasite derivatives and heterozygotes for the PKLR mutation [108, 110].

Finally, it has led to establish that infected erythrocytes under this condition had a greater phagocytic phenomenon related to the development and deposition of hemichromes, IgG, and complement C3c [111, 112].

## 7. Conclusions

Malaria for years has been a study approach for scientists in the approach to the structural and functional study of the constituent proteins of the etiological agent, *Plasmodium falciparum*. A description of important proteins of the parasite has been established, as well as an approach of the main experimental studies that try to explain the molecular basis of each of the main erythrocyte polymorphisms shows a direct and significant resistance against the development of the parasite, and in this way, structural supports and detailed knowledge of some of these polymorphic modifications that show a complete field of study that will lead to the increasingly broad development of new tools for the compression and search for new pharmacological therapies are provided.

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## Conflict of interest

None.

## Acronyms and abbreviations

AMA1	apical membrane antigen-1
CAT	catalase
CR1	complement receptor 1
C3c	complement component C3c
DBL	Duffy binding-like proteins
EDV	electron-dense vesicles
G6PD	glucose-6-phosphate dehydrogenase
GPI	glycosyl-phosphatidyl-inositol protein
GPX	glutathione peroxidase
GR	glutathione reductase
6PG	6-phosphogluconate dehydrogenase
GSH	glutathione reduced
GSSG	glutathione oxidized
HbAS	hemoglobin AS
IgG	immunoglobulin G
MC	Maurer's clefts
KHARP	Knobs-proteins rich in histidines
mi-RNA	micro-ribonucleic acid
MSP1	merozoite surface protein 1
NADP	nicotinamide-adenine dinucleotide phosphate
PV	parasitophorous vacuole
PVM	parasitophorous vacuole membrane
PfEMP1	<i>Plasmodium falciparum</i> erythrocyte membrane protein 1
PKLR	pyruvate kinase isozymes R/L
PK	pyruvate kinase
ROS	reactive oxygen species
RBP	reticulocyte binding proteins
SCT	sickle cell trait
TVN	tubulovesicular networks

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