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Review Article

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Pregnancy-Associated Malaria: Adhesion to Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine and Impact on Genetics of Drug Resistance Markers

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Abstract

Cervical cancer is the only tumor using clinical staging which is continuously improved. The latest 2018 FIGO Malaria remains a major public health issue and one of the main causes of morbidity and mortality in many countries worldwide. Pregnant women are particularly concerned by this disease. To overcome this plague several African countries have adopted intermittent preventive treatment with Sulfadoxine-Pyrimethamine (IPT-SP). Studies led in several countries have shown a strong adhesion to IPTp-SP, from 59% to 94.81%. This adhesion seems to have led to the emergence of Pfdhfr (P. falciparum dihydrofolate reductase) and Pfdhps (P. falciparum dihydropteroate synthase) markers involved in SP resistance at very high levels. The strong prevalence of triple mutant I51R59N108 on Pfdhfr and double mutants G437E540 and G437S581S on Pfdhps increase the risk of therapeutic failure after treatment with SP, as do a high prevalence of haplotype Pfdhfr IRNI and haplotypes Pfdhps SGEAA and the quintuple mutant IRNI-SGEAA. The efficacy of SP could be compromised in countries in which the prevalence of Pfdhfr K540E is higher than 95% and the prevalence of Pfdhps A581G is higher than 10%.

Keywords: Malaria; pregnant; IPTp-SP; resistance markers; Pfdhfr; Pfdhps

Introduction

Malaria is a deadly parasitic infection in its severe form due to protozoans of the genus Plasmodium which invade red blood cells. This disease remains a major public health problem and one of the main causes of morbidity and mortality in many countries worldwide. The World Health Organization (WHO) estimated a number of 241 million cases of malaria and 627,000 deaths due to this disease in 2020 in the world [1].

Sub-Saharan Africa and India bear the most important part of the global burden of malaria with an estimated 85% of cases, among which 96% of deaths occur in Africa. Despite the intensification of malaria control, malaria remains a public health challenge, especially in children under five years old and pregnant women. In sub-Saharan Africa, intense Plasmodium falciparum (P. Falciparum) transmission area, the individuals acquire protective immunity to malaria around the sexual maturity. Pregnant women are an exception to this rule and become even more susceptible to the disease, especially if they are primigravidae, with parasite load often higher than in multigravida who naturally acquire resistance to P. falciparum that reduces parasite density and prevents disease [2, 3].

Indeed, in addition to being more likely to be bitten by malaria vectors [4], the alterations in some immune functions that allow women to tolerate the fetus, which carries 50% of the father's genetic material, make pregnant women more susceptible to infections, especially infections due to intracellular pathogens. The transient depression of cell-mediated immunity - important for the development of immunity to malaria - during pregnancy may explain at least partly the higher susceptibility of pregnant women to malaria compared to non-pregnant women. The hormonal changes such as cortisol, a steroid hormone involved in immunoregulation by suppressing immune system, also promotes increased susceptibility to malaria during pregnancy. [5, 6]. Pregnancy-associated malaria,

which can affect both mother and fetus, constitutes a major cause of maternal and neonatal morbidity such as severe maternal anemia, intrauterine growth retardation, intrauterine death, low birth weight, premature delivery, and stillbirth, and can result in maternal or neonatal mortality [7]. Even when malaria is not the direct cause of death, it can be a cause of co-morbidity, for example with eclampsia . These risks related to malaria during pregnancy can vary according the transmission area and it features. in stable and intense transmission areas, pregnant women are more likely to be infected than those living in low transmission areas, with a greater susceptibility among primigravidae than among multigravida [8]. In addition, in stable transmission area, women are semi-immune and infections are often asymptomatic [9]. In contrast, in low and/or unstable transmission areas, women are rarely infected, and therefore develop low antimalarial immunity [8]. Thus, P. falciparum infections are invariably symptomatic. Untreated, the disease often progresses more quickly to serious disease syndromes. During P. falciparum infection, pregnant women, especially those living in low-transmission areas, are at higher risk of severe malaria and death than their non-pregnant counterparts.[8, 10].

Pathophysiology of pregnancy-related malaria

One of the key points of pregnancy-related malaria is the sequestration of P. falciparum at the placental level, allowing it to escape splenic clearance, as shown by Figure 1 [11]. Previous observations in the increase of phagocytic cells, such as monocytes in the intervillous space, as well as hemozoin deposits would confirm this hypothesis [12]. The analysis of plasmodial populations found in the placenta revealed that parasites adhere to the syncytiotrophoblast covering of the embryonic placental villi via a receptor, « chondroitin sulfate A » (CSA) [11, 13]. The parasite ligand of this sequestration is P. falciparum Erythrocyte Membrane Protein-1 (PfEMP1).

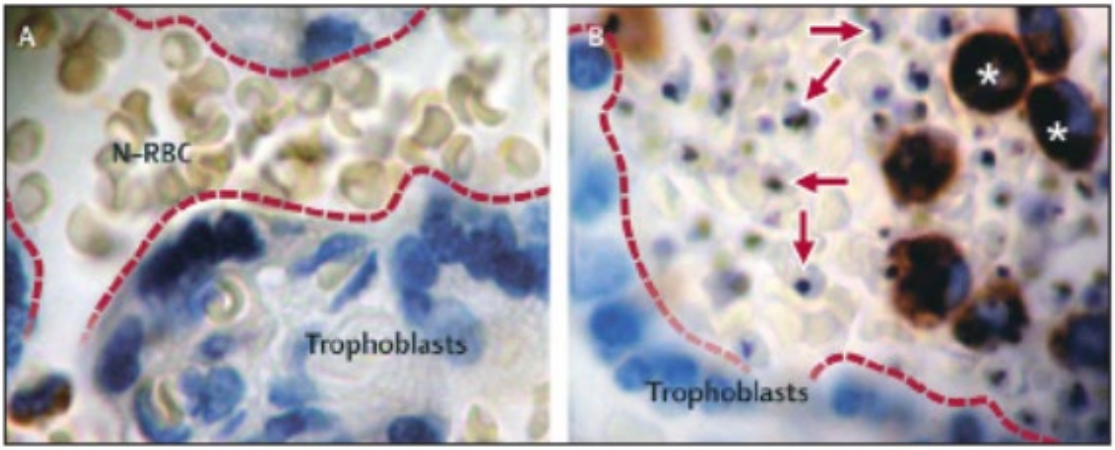

Figure 1: Placental tissue from noninfected (A) and malaria-infected (B). N-RBC, normal red blood cells; red arrows represent parasitized red blood cells.

Ex vivo desequestration experiments have strengthened this point of view. Indeed, injecting Chondroitin Sulfate A (CSA) in infected placentas can recover parasites whose sequestration phenotype adheres to CSA [14]. Other mechanisms such as non-specific binding of parasitized red blood cells on IgM could also be involved in placental sequestration, whereas rosetting mechanisms and auto-agglutination of red blood cells responsible for cerebral sequestration do not seem involved [15]. Genetic analysis of sequestering parasites at the placental level reveals that these parasites express the PfEMP1 variant var2CSA gene [16]. The conservation of this expression would partly explain the development of a specific immunity acquired with parity. Indeed, it is rather well admitted that sera of pregnant women are capable of inhibiting in vitro the cytoadherence of parasitized red blood cells of the CSA phenotype whereas sera of other individuals are unable to [17]. This inhibition is dependent on parity and the sera of primigravida women are as ineffective as those of men to suppress cytoadherence on CSA. Furthermore, antibodies directed against CSA-binding parasites are pan-reactive on placentas infected by P. falciparum, and a high plasma levels of immunoglobulin G (IgG) anti-var2CSA, decrease risk of delivering low birthweight babies [18, 19]. IgG plays an important role, especially in the activation of natural killer cells (NK) and complement, which are the primary mediators of innate immune response and protection against placental malaria [20, 21].

NK cells are the primary mediators of antibody-dependent cell cytotoxicity (ADCC) for the destruction of infected erythrocytes. Their activation also induces the secretion of certain cytokines (gamma interferon and tumor necrosis factor) that help to reduce parasitemia during the early phase of infection, but whose the overproduction contributes to the adverse outcomes of birth associated with malaria during pregnancy [22]. Excessive activation of the complement in placental malaria also contributes to adverse birth outcomes. Elevated levels of C5a in peripheral and placental blood in women with placental malaria have been associated with deregulated angiogenesis in the placenta [23].

From an immunological perspective, pregnancy induces a disequilibrium of the TH1/TH2 balance in favor of the TH2 response. Indeed, increased TH1 responses by circulating T-cells during pregnancy are associated with anemia, miscarriage and premature birth [24, 25]. TH2 response is less effective against P. falciparum and would thus favor the development of parasites during pregnancy.

Finally, from a hormonal point of view, pregnancy-related malaria is associated with a decrease in estradiol rate and an increase in serum cortisol levels which could favor the development of parasites [5].

Intermittent preventive treatment with sulfadoxine-pyrimethamine

Intermittent preventive treatment (IPT) for malaria in pregnant women consists in a complete therapeutic protocol which administers antimalarial drugs during routine prenatal visits, regardless of the presence or not of such an infection in the patients. The WHO recommends IPT-SP in all areas of Africa where the transmission of malaria is moderate to severe. Since 2012, the WHO recommends administering this preventive treatment to all pregnant women in prenatal visits at the beginning of the second trimester of pregnancy. Each woman should at least receive three doses of sulfadoxine-pyrimethamine during pregnancy, at a one-month interval. These can be safely administered until childbirth.

Mechanism of action of sulfadoxine and pyrimethamine

SP is an antimalarial drug combining a compound of the antifolic family, sulfadoxine (SDX) and a compound of the antifolinic family, pyrimethamine (PYR), antimetabolics, and inhibitors of the folic acid biosynthetic pathway of plasmodium [26]. SDX, a structural analog of para-amino-benzoic acid (pABA), is a competitive inhibitor of DHPS, a key enzyme in the folate synthesis pathway, which catalyzes a condensation reaction of pteridine with pABA to form dihydropteroate [27, 28]. PYR is a competitive inhibitor of DHFR, the enzyme that uses dihydrofolate as a substrate to form tetrahydrofolate (THF), an essential co-factor in the biosynthesis of thymidylate and purine bases (Figure 2).These molecules have a long elimination profile, with half-time >80 h, and are active against both the hepatic and erythrocytic asexual stages of the parasite.[29, 30].

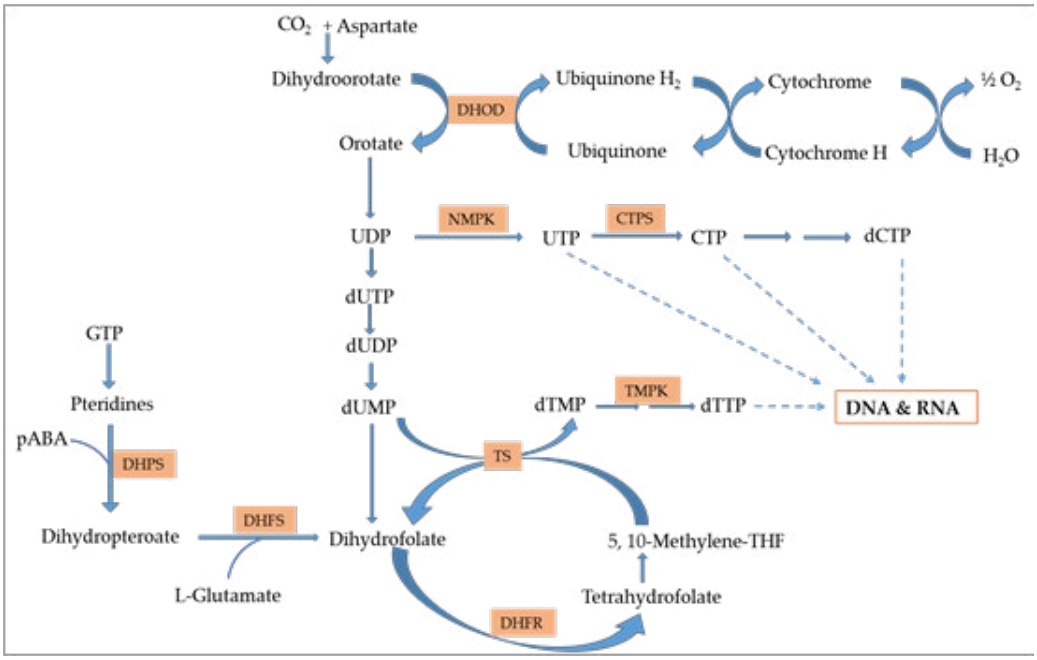

Figure 2: Metabolic pathway of pyrimidine in *P. falciparum* (modified from Le Bras, 1999) [28].

DHOD, dihydroorotate dehydrogenase; DHPS, dihydropteroate synthase; TMPK, thymidylate monophosphate kinase; NMPK, nucleoside monophosphate kinase; CTPS, cytidine triphosphate synthase; TS, thymidylate synthase; DHFR, dihydrofolate reductase; DHFS, dihydrofolate synthase; PABA, para-aminobenzoic acid; UDP, uridine diphosphate; UTP, uridine triphosphate; CTP, cytidine triphosphate; dUTP, desoxyuridine triphosphate; dUDP, deoxyuridine diphosphate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

Adhesion to IPT in Africa

Since the Abuja Declaration of April 2000 on access to effective malaria prevention and treatment for pregnant women in endemic areas, IPT-SP has been adopted by many sub-Saharan African countries to reduce maternal and neonatal morbidity and mortality associated with malaria in pregnancy. Currently, WHO recommends a least two doses of IPT with SP for all pregnant women living in areas of stable malaria transmission. In Africa, a high level of adhesion to this treatment is observed.

In Gabon, a study carried out between 2003 and 2006, assessing the impact of IPTp on maternal and neonatal health, already showed a high adherence of 57% to the newly established national IPTp program of at least two doses by 2005 [31]. This high adherence to IPTp-SP resulted in a dramatic decrease from 10.5% in 2004 to 1.7% in 2006 in the prevalence of maternal P. falciparum infection in both urban and rural areas after only two years of implementation of the new program. Marked benefits were also observed on the prevalence of low birth weight and preterm births [31].

In Ghana, studies have also reported high level of adhesion to IPTp-SP only one to two years after its adoption and implementation as national policy [32, 33]. In Agogo, Southern Ghana, in 2006, 77% of women reported taking IPT-SP at least once [32]. In South-west, in Offinso District, another study in 2007 reported that 85% of pregnant women recruited had taken at least one dose, and among them, 70% had taken at least two doses [33]. In addition, SP use reduced pregnancy-related malaria, the prevalence of maternal anemia, maternal morbidity, and contributed to increased birth weight of newborns.

In other African countries such as Kenya or Burkina Faso, similar results were reported [34, 35]. Countries such as Sierra Leone report a prevalence of IPT-SP up to 94.81% [36, 37]. In Zambia IPT-SP has been shown to have a protective effect against malaria during pregnancy, especially in primigravida women [37]. However, in Malawi, IPT-SP only maintained a partial activity in pregnant women presenting asymptomatic malaria [38].

Association of IPTp-SP and sulfadoxine pyrimethamine resistance

Despite the efficacy of IPT-SP, there is a growing concern in face of the increase in resistance to this treatment. Studies highlighted that resistance to SP is associated with mutations on two targets considered as markers: Pfdhfr and Pfdhps.

Pfdhfr

This protein is coded by the Pfdhfr gene, located on chromosome 4. This gene is concomitant to Thymidylate-synthase. The mutation of serine into asparagine on codon 108 (S108N) is the most predominant for resistance to pyrimethamine, in vitro [39]. Then, it was shown that the addition of mutations on other codons, 50, 51, 59 and 164 increases the resistance level of P. falciparum. Thus, the double mutations S108+N51I or S108N+C59R increase the inhibitory concentration of parasites by 50% (IC50), between 2 to 16 times compared to the simple mutant S108N [40, 41]. Triple mutants (N51I+C59R+S108N and C59R+S108N+I164R) express a higher resistance level to that of double mutants while quadruple mutants are even more resistant than triple mutants [42, 43].

IRNI triple mutants are predominant in several countries. In Lagos, Nigeria, the rate of appearance of Pfdhfr 108N was 73.3%, in 2015 [44]. In Tanzania, a study led in 2013 highlighted CIRNI triple mutant haplotypes which were predominant in all the sites with very high frequencies [45]. In Burkina Faso in 2010, a study showed that mutations C59R and S108N were the most common with prevalence rates of 61.2% and 55.7%, respectively, as well as a low prevalence of mutation N51I (12.2%). The prevalence rate of NRNI double mutations et des IRNI triple mutations were 35.7% and 11.4%, respectively [46]. Although mutations in Pfdhfr genes are relatively common, the prevalence of triple mutations is very low, indicating that IPT-SP is always efficient in Burkina Faso [46].

After implementing IPT-SP, a strong apparition of resistance genotypes is observed. This could lead to a decrease in the efficacy of SP. In Ghana, a study led on pregnant women reported that the prevalence of IRNI triple mutant haplotypes was high at childbirth among the post-SP treatment isolates (18.2%) compared to those of the first prenatal care (6.1%) before initiating IPT-SP during pregnancy [47]. Similarly, in Gabon, it was reported that the prevalence of triple mutant Pfdhfr had significantly increased between the implementation of IPT-SP in 2005 and six years after in 2011 [48].

In Uganda, a study performed from samples collected between 2004 and 2008 reported that before IPT-SP, the prevalence rate of the five most common antifolate mutations was higher than 92% and that this prevalence had increased significantly after exposure to SP [49].

In Burkina Faso it was shown that the use of IPT-SP was associated with a threefold increase of the probability of mutation Pfdhfr C59R because pregnant women who had recently adopted IPT-SP presented higher rates of this mutation [50].

Pfdhps

As for Pfdhfr, the dihydropteroate synthetase (DHPS) is an enzyme of the folate synthesis pathway (Figure 2). It is coded by a gene located on chromosome 8 of P. falciparum. Mutations on codons 436, 437, 540, 581 and 613 have been associated with resistance to sulfadoxine: these lead to a reduction in the binding affinity of sulfadoxine on its target [51]. Mutation A437G increases IC50 by almost 5 times [52]. Even though the accumulation of mutations is not directly linked to an increase in the resistance level, it has been shown that triple mutants S436A+A437G+K540E and S436A+A437G+A613S increase IC50 by 9.8 and 24 times, respectively, compared to the wild strain [53].

Triple mutant I51R59N108 on Pfdhfr and double mutants G437E540 and G437S581S on Pfdhps increase the risk of therapeutic failure after a treatment with SP [54]. The selection of super-resistant Pfdhps to SP A581G is higher in the north of Tanzania [55].

The efficacy of SP to eliminate peripheral parasites and the prevalence of new infections during pregnancy is compromised in areas where the prevalence of Pfdhps K540E is higher than 90%. Nevertheless, in these high resistance areas, the use of IPT-SP is still associated to an increase of weight in infants at birth and maternal

hemoglobin [56].

In Cameroon, during a study led in 2015, it was shown that the most frequent Pfdhps mutation was A437G with a 76.5% prevalence rate in pregnant women who had not taken IPT-SP, which is significantly higher than in women who had taken IPT-SP (95.9%) [57]. The prevalence of mutations Pfdhps A581G and A613S has increased since the last studies in 2005. Mutation Pfdhps K540E was detected but remains rare. Moreover, the new mutation Pfdhps I431V was also detected with a 9.8% prevalence. In Congo we find mutants Pfdhps 436A, 437G and 540E. Quintuple mutations with the highly resistant haplotypes Pfdhps 437G/540E were detected in more than half the collected isolates [58].

A constant increase in the prevalence of triple-mutant genotype H436-G437-E540: A581-A613 was found in the west of Kenya between 2010 and 2013 in children suffering from uncomplicated forms of malaria [59].

In a study led in Malawi, in a total of 202 specimens genotyped in codon 581 of the DHPS gene, 8.4% harbored the sextuple mutant [38].

In Ghana, a study including pregnant women reported that the prevalence of Pfdhps A581G mutants was high at childbirth among the isolates of post-SP treatment (18.2%) compared to those of the first antenatal care (before initiating IPT-SP during pregnancy) 6.1% ; p=0.03 [47]. The presence of Pfdhfr/Pfdhps quintuple mutant N51I/C59R/S108N/A437G/K540E should also be noted since it is the most medically resistant marker to SP [60, 61].

Genotypes associated with therapeutic failure

A study led in Congo between 2012 and 2013, seven years after implementing IPT-SP, shows high levels of SP-resistant mutations in isolates of P. falciparum collected in pregnant women as well as the presence of the mutant allele Pfdhps 540E and the strong prevalence of isolates carrying Pfdhfr/Pfdhps quintuple mutations reported here for the first time in the Republic of the Congo [58].

In Tanzania, a study from 2020 shows that the IRNI haplotype was predominant with 84% and that the double haplotype Pfdhps SGEAA represented 83% of all the mutations in gene Pfdhps. The quintuple mutant SGEAA-IRNI represented 71.4%. The global prevalence rate of Pfdhfr S108N was 90.4% and that of Pfdhps A581G was 1.1% [62].

A study led in the west of Kenya in 2010 and 2013 reveals that the presence of triple mutant H436-G437-E540:A581-A613 can increase in pregnant women and could compromise the efficacy of SP for IPT if it further increases the resistant threshold [59].

Studies led in Malawi between 2009 and 2011 reported that 8.4% of genotyped samples expressed the sextuple mutant. It was associated with high risks of patent infection in the peripheral blood and placental blood. Since IPT-SP has failed to inhibit the increase of parasites, new measures to prevent malaria in pregnant women must be considered [38].

The efficacy of SP to eliminate peripheral parasites and the prevalence of new infections during pregnancy is compromised in areas in which the prevalence of Pfdhps KS40E is higher than 90% [56, 63]. It is now crucial to explore other effective antimalarial drugs as an alternative to SP for IPTp so as not to compromise malaria control and prevention in pregnant women.

Conclusions

The presence of SP resistance markers at high levels and that of the quintuple mutant IRNGE threaten the future of SP in IPT-SP programs and as a combination drug for ACT [60]. It is necessary to explore at a larger scale adhesion to IPT, genotypes associated with SP resistance and the efficacy of IPT, especially in rural areas for which there is a lack of information Our current and even past work here in Gabon is consistent with this need, in particular with the aim of providing sufficient data and information on the level of adhesion of pregnant women to IPT-SP in different contexts (rural, urban, semi-urban), as well as on the prevalence levels of molecular markers associated with resistance to SP across the country.

Abbreviations: IPTp-SP: intermittent presumptive treatment for pregnant women with Sulfadoxine-Pyrimethamine; CSA, chondroitin sulfate A; Pfdhfr, Plasmodium falciparum dihydrofolate reductase; Pfdhps, Plasmodium falciparum dihydropteroate synthase

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