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Clinical Pharmacology of the Antimalarial Chloroquine in Children and Their Mothers

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Abstract

Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi are the parasites that infect humans. Plasmodium falciparum and Plasmodium vivax cause most of the malarial infections worldwide. Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi are susceptible to chloroquine. Chloroquine was the world's most widely used antimalarial drug, but the most common and virulent parasite Plasmodium falciparum is now increasing resistance. Chloroquine-sensitive Plasmodium falciparum concentrates chloroquine to higher levels than did chloroquine-resistant parasite. Chloroquine concentrates in the highly acidic digestive vacuoles of susceptible Plasmodium parasites, where it binds to heme and disrupts its sequestration. Failure to inactivate or even enhanced toxicity of drug-heme complexes kill parasites via oxidative damage to membranes and digestive proteases.

The loading dose of chloroquine in children is 10 mg/kg administered intravenously or by mouth and then three 5 mg/kg doses of chloroquine every 24 hours starting 6 hours after the loading dose should be given. Chloroquine is well absorbed, widely distributed in body tissues, slowly metabolized by the liver and very slowly cleared from the body. Residents in malaria endemic areas develop considerably immunity over time, but pregnancy makes women more vulnerable and infection during pregnancy increases the risk of anemia, miscarriage, stillbirth, and prematurity. The children are more vulnerable than adults to malaria infection. The aim of this study is to review the published data on the clinical pharmacology of chloroquine in children and their mothers.

Key Words: Children, Chloroquine, Effects, Pregnant-women, Resistance.

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1-INTRODUCTION

Chloroquine was, for a long time, the world's most widely used antimalarial drug, but the most common and virulent parasite, Plasmodium falciparum is now increasingly resistance. Chloroquine-sensitive Plasmodium falciparum concentrates chloroquine to higher levels than did chloroquine-resistant parasite. Chloroquine is well absorbed, widely distributed in body tissues, slowly metabolized by the liver and very slowly cleared from the body. Residents in malaria endemic areas develop considerably immunity over time, but pregnancy makes women more vulnerable, and infection during pregnancy increases the risk of anemia, miscarriage, stillbirth, and prematurity. In 2010, there were an estimated 219 million cases of malaria infection resulting in 660,000 deaths. Approximately two thirds of deaths occurred in children, children are more vulnerable than adults to malaria infection. In sub-Saharan Africa, maternal malaria infection is associated with up 200,000 estimated infant deaths yearly (1).

Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi are the malarial parasites that infect humans. Plasmodium falciparum and Plasmodium vivax cause most of the malarial infections worldwide. Plasmodium falciparum is the most common and virulent parasite and is often resistant to chloroquine. Plasmodium falciparum accounts for the majority of malaria infection in sub-Sahara Africa and is associated with most severe disease. Plasmodium vivax accounts for half of the malarial infection in South and East Asia and > 80% of the malarial infections in the America (2). Chloroquine, a weak base, concentrates in the highly acidic digestive vacuoles of susceptible Plasmodium parasites, where it binds to heme and disrupts its sequestration. A parasite-encoded efflux mechanism may account for the reduced levels of chloroquine in the digestive vacuoles of chloroquine-resistant parasites (3). The ability to treat and control Plasmodium falciparum infection through chemotherapy has been compromised by the advent and spread of resistance to antimalarial drugs. Plasmodium falciparum chloroquine resistance transporter (PfCRT), and the drug multidrug resistance-1 (pfmdr1) transporter as key determinants of decreased in-vitro susceptibility to several principal antimalarial drugs. Transfection-based in-vitro studies are consistent with clinical findings of an association between amplification of the pfmdr1 gene and failure of mefloquine treatment (4).

Resistance to Plasmodium parasites is conferred by mutation in pfcrt, an integral membrane protein localized to the parasite's internal digestive vacuole. These mutations result in a marked reduction in the accumulation of chloroquine by the parasite (3). At present there is no effective malaria vaccine and malaria is totally reliant on the use of drugs. New drugs are urgently needed because of the rapid evolution and spread of parasite resistance. Drug resistance is one of the major factors contributing to the resurgence of malaria, especially resistance to the most affordable such as chloroquine. It is important to fully understand the antimalarial mode of action of the existing drugs and the way that the parasite becomes resistant to them in order design and develop new therapies that are so urgently needed (5).

2- MATERIALS AND METHODS

2-1. Literature Search

The following databases were searched for relevant papers and reports: MEDLINE via (PubMed), CINAHL, EMBASE, Google scholar as search engines; February 2018 was the cutoff point. Key references from extracted papers were also hand-searched.

2-2. Search Terms

The following key words "chloroquine neonates", "chloroquine effects neonates", "chloroquine pharmacokinetics neonates", "chloroquine metabolism neonates", and "chloroquine resistance neonates" were used. In addition the book Neonatal Formulary (1) was consulted.

3-RESULTS

3-1. Mechanism of action of chloroquine

Hemoglobin degradation by Plasmodium parasites is a massive catabolic process within the parasite food vacuole that is important for the organism's survival in its host erythrocyte. A proteolytic pathway is responsible for generating amino acids from hemoglobin. Each of the enzymes involved has its own peculiarities to be exploited for development of antimalarial agents that will starve the parasite or result in build-up of toxic intermediates (6).

3-2. Dosing of chloroquine in children

Prevention in visitors: offer children 5 mg/kg of chloroquine base by mouth once a week in areas where sensitive parasites are endemic. Start 1 week before entering the area and stop 4 weeks after leaving. Consider giving proguanil (q.v.) as well (1).

Cure: give a 10 mg/kg loading dose of chloroquine base intravenously or by mouth and then three 5 mg/kg doses of chloroquine (given at 24 hour intervals) starting 6 hours after the loading dose was given. In Plasmodium vivax or Plasmodium ovale infections, there may be a case for giving primaquine after this to eliminate any dormant liver parasites (1).

3-3. Effects of chloroquine in children

Thirty-five children with Plasmodium malaria infection were treated with 25 mg/kg chloroquine over three days and observed for seven days during which blood films were examined daily for malaria parasites (7). Asexual forms of Plasmodium falciparum which were present in the blood films of all patients before commencing treatment disappeared rapidly from the blood so that by the third day no parasites were seen in the blood film. The blood films remained negative for the rest of seven-day observation. Plasma chloroquine determination in eight of the patients showed high blood levels during the first three days. The parasitological, clinical, and hematological response to chloroquine treatment was studied in children during a 28-day follow-up in an area of Côte d'Ivoire with intermediate chloroquine resistance (8). The parasitological, clinical, and hematological response to Fansidar was also investigated in patients who returned to the health centre within 28 days with symptoms of malaria infection. Of 82 children aged between 0 to 9 years who completed the study, only 67% were parasite-negative on thick blood film on day 7, which decreased to 21% by day 28; while chloroquine treatment still produced clinical remission at day 7 in 95% of the children, 35% had recurrent fever with concomitant parasitaemia before day 28. All fever cases subsequently treated with Fansidar remained parasite-negative over a period of 28 days.

On day 28 the hematocrit levels were higher in those children who responded successfully with either chloroquine or Fansidar than in the children who were still parasite-positive but without fever (two-tailed test, $p = 0.02$). The rate of resistance to chloroquine was most pronounced among the younger children (< 5 years old), 18% of whom showed clinical failure by day 14. These present findings underline the importance of monitoring the durability of response to chloroquine treatment for at least 14 days in young children in Côte d'Ivoire.

Chronic use of chloroquine and hydroxychloroquine in the treatment of rheumatic disease carries a small risk of sight-threatening pigmentary retinopathy. To obtain safety data for its use in pregnancy, Klinger et al. (9) did ophthalmic examination in 21 children born to women who took these drugs during pregnancy. Average daily maternal doses of the two drugs were 317 mg hydroxychloroquine and 332 mg chloroquine. The mean duration of gestational exposure was 7.2 months. No ophthalmic abnormality was detected in these children. Therapeutic use of chloroquine and hydroxychloroquine during pregnancy may not pose a significant risk of ocular toxicity to offspring. In spite of increasing resistance, chloroquine remains the primary drug for treatment of malaria in most sub-Saharan African countries.

Zucker et al. (10) evaluated the effect of drug treatment policy on the case-fatality rates of children, adjusting for differing distributions of malaria and severe anemia. In 1991, 63% of children were treated with chloroquine while the remaining 37% were treated with a regimen that would eliminate and clear parasitaemia. Case-fatality rates were 13% and 4.1%, respectively; the proportion of deaths attributable to chloroquine treatment was 69%. The trend in case-fatality rates for malaria infection decreased as an increasing proportion of children received an effective treatment regimen; adjusted malaria case-fatality rates were 5.1%, 3.6%, and 3.3% in 1992, 1993, and 1994, respectively, when 85% of children in 1992 and 97% of children in 1993-1994 received effective therapy. These 4 years of data provide strong evidence that continued use of chloroquine in areas with resistance contributing to excess Plasmodium falciparum-related deaths. The role of chloroquine as a first-line drug to treat Plasmodium falciparum is almost universally becoming questionably. Kebede et al. (11) conducted in one of the country's unstable malaria endemic area, North Shoa with the objective of assessing the in-vivo treatment efficacy of chloroquine to Plasmodium falciparum malaria infection using the standard World Health Organization (WHO) 14 days treatment response monitoring guideline. A total of 427 patients were followed among which 87.8% shoved treatment failure. This was more pronounced in children than in adults (Chi-square for trend = 8.16; $p < 0.01$). Clinical presentation with high grade fever on day 0 was found to be more predictive of treatment failure in children (odds ratio = 2.06; 95% confidence interval = 1.26-3.36; $p < 0.005$). Tendency to remain febrile on subsequent follow-up days was also more observed in children compared to adults.

Treatment failure was further associated with high Parasite Density Index on day 0 in all age groups (odds ratio = 1.99; 95% confidence interval = 1.04-3.83; $p < 0.05$). Supplemented with large scale sensitivity studies, it is high time that switch to alternate drugs needs due consideration by policy makers. Over 2 million children globally are human immunodeficiency virus (HIV) positive. More than 90% are infected in uterus from their mothers. Current pharmacological methods to reduce the rate of vertical transmission are too expensive for the developing world. Chloroquine, a cheap, widely available drug, has anti-HIV properties. Neely et al. (12) conducted a pilot study to determine if chloroquine can reduce HIV vertical transmission. A total of 287 samples of cord blood from a cohort of Uganda infants born to HIV positive mothers were analyzed for concentrations of chloroquine and its two major metabolites, monodesethylchlorooquine and didesethylchloroquine. The HIV status of each infant was determined by ELISA with Western Blot confirmation at 15 and 18

months of age. A percentage of 49 of samples had measurable chloroquine or metabolites. Of those with measurable drug, the higher concentrations of chloroquine and its metabolites were more frequently associated with HIV negative infants. However, only the median concentration of didesethylchloroquine was significantly higher in HIV negative infants versus HIV positive infants (1.6 ng/ml versus 0.9 ng/ml; p = 0.05). Nearly half of infants in a Uganda cohort are exposed to chloroquine in the last trimester of pregnancy. Such random maternal chloroquine use may be associated with a decrease rate of HIV vertical transmission. The issue of maternal chloroquine use requires controlled study before any clinical conclusion may be drawn.

3-4. Treatment of chloroquine in children

Plasmodium falciparum malaria is a significant problem for Afghan refugees in Pakistan. Refugee treatment guidelines recommended standard three-day chloroquine treatment (25 mg/kg) for first episodes and extended five-day treatment (40 mg/kg) for recrudescent infection, based on the assumption that a five-day courses would more likely achieve a cure (13). An in-vivo randomized controlled trial was conducted among refugees with uncomplicated Plasmodium falciparum malaria infection to determine whether five-day treatment of 40 mg/kg chloroquine was more effective than standard treatment of 25 mg/kg chloroquine. A total of 142 Plasmodium falciparum patients were recruited into 25 mg/kg chloroquine or 40 mg/kg chloroquine arms and followed up to 60 days with regular blood smears. The primary outcome was parasitological cure without recrudescence. Treatment failures were treated with 40 mg/kg chloroquine. PCR genotyping of 270 samples, from the same and nearby sites, was used to support interpretation of outcomes. A percentage of 84 patients treated with 25 mg/kg chloroquine versus 51% of patients treated with 40 mg/kg chloroquine experienced parasite recrudescence during follow-up (adjusted odds ratio = 0.17; 95% confidence interval = 0.08-0.38). Cure rates were significantly improved with 40 mg/kg chloroquine, particularly among adults. Fever clearance time, parasite clearance time, and proportions gametocytaemic post-treatment were similar between treatment groups. Second-line of 40 mg/kg chloroquine treatment resulted in higher failure rates than first-line 40 mg/kg chloroquine treatment. Chloroquine-resistance marker pfcrt 76T was found in all isolates analyzed, while pmdr1 86Y and 184Y were found in 18% and 37% of isolates, respectively. Chloroquine is not suitable for first-line falciparum treatment in Afghan refugee communities. The extend-dose of chloroquine regimen can overcome 39% of resistant infections that would recrudescence under the standard regimen, but the high failure rate after directly observed treatment demonstrates its use is inappropriate.

Malaria continues to be a leading cause of morbidity and mortality in children aged 5 years or younger in Tanzania. Children who develop mild disease can rapidly progress to severe malaria (cerebral malaria with convulsions), and even death, because of mismanagement, delays and inappropriate drug therapy in the remote areas where primary health care facilities are inaccessible or unavailable. The threat is particularly severe in those who are unable to take oral medications. Nsimba and Rimoy (14) identified treatment strategies adopted by mothers or guardians of children under 5 years old for malaria infection. Over 500 mothers/guardians of sick children aged up to 5 years who visited the public facilities seeking care were interviewed in order to assess what management they offered to their sick

children in their homes prior to coming to the public health facilities. Seventy-four per cent of the mothers/guardians stated that they had given some medication to their children prior to visiting the public health facilities: mostly analgesic (aspirin, paracetamol) and chloroquine. Eight-five per cent of the sick children given chloroquine had whole blood chloroquine levels above 500 nmol/l and 33% of the sick children with whole blood chloroquine levels above 1,000 nmol/l had malaria parasites in their blood. Of the sick children given chloroquine at the health facilities, 63% had no malaria parasites in their blood. There is a need to educate both rural communities, and health care providers about rational prescribing, dispensing and use of antimalarials.

3-5. Comparative efficacy of chloroquine with other antimalarial drugs in children

Chloroquine remains the first-line treatment for uncomplicated malaria infection in much of Africa despite the growing problem of resistance to this drug. Sulfadoxine-pyrimethamine is often used after chloroquine treatment failure and has replaced chloroquine treatment failure as the first-line treatment in parts of Africa. To compare the efficacy of these 2 regimens, Kamya et al. (15) evaluated, in March-August 1999, clinical and parasitological responses over 28 days in 214 children and adults from Kampala, Uganda, with uncomplicated malaria. Compared to sulfadoxine-pyrimethamine, significantly more patients treated with chloroquine developed early or late clinical failure (54% versus 11%, $p < 0.001$) and parasitological failure (72% versus 30%; $p < 0.001$) during 14 days of follow-up. The risk of treatment failure occurring after day 14 was similar between the 2 treatment groups. Among those treated with chloroquine, children aged < 5 years were at higher risk of clinical failure than older individuals (76% versus 28%, $p < 0.001$), an association not seen with sulfadoxine-pyrimethamine (11% versus 10%, $p = 0.91$). Although early parasite clearance was significantly better in the sulfadoxine-pyrimethamine group ($p = 0.001$), fever clearance at day 3 was the same (chloroquine 85%, sulfadoxine-pyrimethamine 86%). The present findings suggest that consideration be given to replace chloroquine as the first-line therapy for uncomplicated malaria infection in Uganda, particularly in young children. Resistance of Plasmodium falciparum to chloroquine is widespread in Papua New Guinea. At meeting in Port Moresby in October 1997, it was decided to explore a possibly change of the current first-line treatment of uncomplicated malaria with chloroquine alone (amodiaquine for children under five years) or amodiaquine in combination with sulfadoxine-pyrimethamine (16). To assess the therapeutic efficacy of the new drug combination in Papua New Guinea, a study was carried out in 1998-1999 at five hospital outpatients departments. From 513 children enrolled in the study, 95 defaulted from follow-up.

Of the remaining 418, 399 (95.5%) had an adequate clinical response. Out of the 19 patients who did not have an adequate clinical response, 3 (0.7% of the total) developed severe signs in the first 24 hours and were treated in hospital; they were regarded as early treatment failures. The remaining 16 did not complete the study on the basis of various exclusion criteria but were not excluded from the analysis. From these results it was concluded that the combination was effective and a decision was taken in May 2000 to introduce the two-drug combination regimens as the standard first-line treatment of uncomplicated malaria, including falciparum malaria, in Papua New Guinea. Few studies have documented the effectiveness in West Africa of intermittent preventive treatment

of malaria with sulfadoxine-pyrimethamine in pregnancy (17). Pregnant Nigerian women were assigned to receive either sulfadoxine-pyrimethamine given twice or presumptive chloroquine treatment followed by weekly pyrimethamine; 250 pregnant women were enrolled in each group. Of these completing follow-up, 4 (1.8%) in the sulfadoxine-pyrimethamine group and 22 (9.8%) in the chloroquine plus pyrimethamine groups had a febrile illness ($p = 0.005$). None in the sulfadoxine-pyrimethamine group but 11 (4.9%) in the chloroquine plus pyrimethamine group had peripheral parasitaemia prior to or during delivery ($p = 0.002$). There were six low-birth-weight infants in the sulfadoxine-pyrimethamine group and eight in the chloroquine plus pyrimethamine group ($p = 0.21$). Intermittent preventive treatment with sulfadoxine-pyrimethamine is superior to chloroquine plus pyrimethamine for prevention of malaria infection and anemia in pregnant women in Nigeria.

Following the WHO protocol for in-vivo tests in areas with intense transmission of uncomplicated Plasmodium falciparum malaria infection, a randomized comparison of the in-vivo efficacy of chloroquine alone, sulfadoxine-pyrimethamine alone, and their combined administration was carried out in the third quarter of 2001 in Kaberamaido District, northeastern Uganda (18). Malaria in the study area is hyper-endemic, with a high prevalence of Plasmodium falciparum. The patients were infants and young children with a median age of 15 months. Of the 117 originally enrolled children, 104 had a complete follow-up with presentation at all scheduled examinations. In the chloroquine group ($n = 42$), 55% were classified as adequate clinical response, 26% as early treatment failure, and 19% as late clinical failure. In the sulfadoxine-pyrimethamine group ($n = 30$), the respective figures 83%, 13%, and 3%, respectively, and in the group with combined treatment ($n = 32$), were 88%, 6%, and 6%, respectively. In terms of clinical cure rate, speed of clinical relief and parasite clearance the combined treatment proved to be the most effective of the three drug regimens. In the children with adequate clinical response, a significant post-therapeutic increase of the hematocrit was observed, which was particularly marked in children who had also cleared their parasitaemia. Increase of the efficacy of chloroquine with age indicates the early development of semi-immunity in the study area, with conserved efficacy of chloroquine in semi-immune persons. Legros et al. (19) conducted a 14-day study, during March-May 1998, to assess the efficacy of chloroquine and sulfadoxine-pyrimethamine for treating uncomplicated Plasmodium falciparum malaria infection in Uganda. Overall treatment failure rates were 43 (81.1%) of 53 chloroquine recipients and 16 (25.0%) of 64 sulfadoxine-pyrimethamine patients. Strategies to improve the life-span of standard and affordable anti-malarial are needed.

3-6. Treatment of chloroquine and other antimalarial drugs in pregnant women

Malaria in pregnancy is one of the most common preventable causes of maternal and neonatal morbidity and mortality in sub-Saharan Africa. To prevent its adverse effects, such as maternal anemia, placental parasitaemia and low-birth-weight neonates, the WHO recommends effective malaria case management, use of insecticide-treated bed-nets and intermittent preventive therapy in pregnancy. Sulphadoxine-pyrimethamine has been the standard for intermittent preventive therapy in pregnancy in several countries, but parasite resistance to Sulphadoxine-pyrimethamine is growing. Therefore, new intermittent preventive therapies in pregnancy are urgently

needed. One candidate being evaluate for intermittent preventive therapy in pregnancy is a fixed-those combination of azithromycin and chloroquine. Chandra et al. (20) described the challenges and the innovative solutions implemented in designing and conducting a pivotal azithromycin and chloroquine intermittent preventive therapy in pregnancy. The azithromycin and chloroquine intermittent preventive therapy in pregnancy pivotal trial is a multicentre, multicounty, phase III, open-label, randomized superiority study of azithromycin and chloroquine intermittent preventive treatment in pregnancy versus sulfadoxine-pyrimethamine therapy in pregnant women of sub-Saharan Africa. The trial was designed to meet stringent regulatory agency scientific advice and the intermittent preventive therapy in pregnancy policy markers' recommendations, and incorporates an innovative adaptive design to manage programme risk, maintain the operating characteristics of the study and optimize resources.

The trial's novel composite primary endpoint is proportion of participants with a suboptimal pregnancy outcome (abortion [≤ 28 weeks], stillbirths [> 28 weeks], and premature [< 37 weeks] deliveries, low-body-weight [2,500 grams] live neonates, missing neonatal birth data weight data or loss to follow-up). The study employs a prospective group sequential design with three unblended analyses when 50%, 70%, and 100% of participants achieve the primary endpoint; the study team will remain blinded to the analyses until after the completion of the study. The number of participants randomized will be adaptive, based on the blinded review of the observed pooled primary endpoint data across the two treatment arms, when approximately 1,000 participants complete the primary endpoint assessments. These present findings describe the unique challenges and innovative solutions implemented in designing and conducting this pivotal azithromycin and chloroquine intermittent preventive therapy in pregnancy trial, which may serve as a prototype for future intermittent preventive therapy in pregnancy and other studies involving similar conditions. The WHO recommends intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine in Africa regions with moderate to high malaria transmission. However, growing resistance to sulfadoxine-pyrimethamine threatens the effectiveness of intermittent preventive therapy in pregnancy of sulfadoxine-pyrimethamine, and alternative drugs are needed. Kimani et al. (21) tested the efficacy, tolerability, and safety of a fixed-dose combination azithromycin plus chloroquine (250 mg azithromycin and 155 mg chloroquine base) for intermittent preventive therapy in pregnancy relative to intermittent preventive therapy in pregnancy with sulfadoxine-pyrimethamine.

A randomized, phase 3, open-label, multicentre study was conducted in sub-Saharan Africa countries (Benin, Kenya, Malawi, Tanzania, and Uganda) between October 2010 and November 2013. Pregnant women received 3 intermittent preventive therapy courses with azithromycin plus chloroquine (each course: 1,000/620 mg azithromycin plus chloroquine for 3 days) or sulfadoxine-pyrimethamine (each course 1,500/75 mg sulfadoxine-pyrimethamine plus chloroquine 1 day) at 4- to 8-week intervals during the second and third trimester. Long-lasting insecticide-treated bed-nets were also provided at enrollment. Study participants were followed-up until day 28 post-delivery (time window: day 28-42). The primary endpoint was the proportion of participants with sub-optimal pregnancy outcomes (a composite endpoint comprising live-born neonates

with [low-birth-weight < 2,500 grams], premature birth [<37 weeks], stillbirth [> 28 weeks], abortion [≤ 28 weeks], lost to follow-up prior to observation of pregnancy outcome, or missing birth weight). The study was terminated early after early recruitment of 2,891 of the planned 5,044 participants, due to futility observed in a pre-specified 35% interim analysis. In the final intent-to-treat dataset, 378/1,445 (26.2%) participants in the azithromycin plus chloroquine and 342/1,445 (23.7%) in the sulfadoxine-pyrimethamine group had sub-optimal pregnancy outcomes, with an estimated risk (risk ratio = 1.11; 95% confidence interval; p-level = 0.12).

There was no significant difference in the incidence of low-birth-weight between treatment groups (57/1,138 [5.0%] in the azithromycin plus chloroquine group, 68/1,188 [5.7%] in the sulfadoxine-pyrimethamine group, (relative risk = 0.87; 95% confidence interval = 0.62-1.23; p = 0.44). Intermittent preventive therapy in pregnancy of azithromycin plus chloroquine was less well-tolerated in mothers than intermittent preventive therapy in pregnancy of sulfadoxine-pyrimethamine. Occurrences of congenital anomalies, deaths, and serious adverse events were comparable in neonates for both groups. Limitations included the open-label design and early study termination. Intermittent preventive therapy in pregnancy of azithromycin plus chloroquine was not superior to the intermittent preventive therapy in pregnancy of sulfadoxine-pyrimethamine in this study alternatives for intermittent preventive therapy of sulfadoxine-pyrimethamine remain to be identified. The proportions of sub-optimal pregnancy outcomes and low-birth-weight were lower than expected, which may be linked to insecticide-treated bed-nets use throughout the study. Reduced incidences of symptomatic malaria infection and peripheral parasitaemia in the azithromycin plus chloroquine group relative to sulfadoxine-pyrimethamine suggest that azithromycin plus chloroquine warrants further investigation as an alternative treatment of uncomplicated malaria. Despite international recommendations to use malaria treatment in pregnant women in malaria-endemic areas, few studies have evaluated the efficacy of available antimalarial regimens. This issue is of particular concern in the face of spreading chloroquine-resistance of Plasmodium falciparum in malarials areas of sub-Saharan Africa. In a prospective trial in rural Malawian pregnant women, Steketee et al. (22) examined three regimens using chloroquine (including the existing national policy regimen) and one regimen using mefloquine.

The efficacy of the regimens was determined by comparing rates of clearance of initial parasitaemia; prevention of breakthrough infection and parasitaemia at delivery in maternal peripheral blood, placental blood, and in infant umbilical cord blood. Among 1,528 parasitaemic women at enrollment, 281 (18.4%) had persistent infections; and among 1,852 initially parasitaemic women on mefloquine, 320 (17.3%) had breakthrough parasitaemia on one or more follow-up visits. Compared with women on mefloquine, women on a chloroquine regimen were at significantly greater risk of persistent and breakthrough infection (odds ratios = 30.9 and 11.1, respectively, p < 0.001). During September 1987 to June 1990, 3,380 pregnant women with parasitaemia attending 4 prenatal care clinics in rural Mangochi District, Malawi, were assigned to 1 of 4 regimens of antimalarial treatment and/or prophylaxis. The women were followed through delivery to determine the antimalarial drug efficiency on peripheral parasitaemia during pregnancy and parasitaemia at the

time of delivery in peripheral, placental, and umbilical cord blood. Parasite clearance was not achieved in 18.4% of the 1,528 women with parasitaemia at enrollment. A percentage of 17.3 of the 1,852 women were aparasitaemic at enrollment had breakthrough infections. Women using a chloroquine regimen faced a significantly greater risk of persistent and breakthrough parasitaemia (odds ratios = 30.9 and 11, respectively; p = 0.001).

The multivariate analysis found other significant risk factors for malaria to be first pregnancy (odds ratios = 3.6 for persistent malaria and 1.5 for breakthrough malaria), enrollment in the rainy or post-rainy season (odds-ratios = 2-3.4 for persistent parasitaemia and 1.2-2.7 for breakthrough malaria), maternal age of at most 25 years (odds ratios = 2.3 for persistent malaria and 1.6 for breakthrough malaria), and seropositive to HIV (odds ratio = 1.9 for persistent malaria and 1.6 for breakthrough malaria). At delivery, women on a chloroquine regimen faced a significantly higher risk of peripheral, placental, or umbilical cord parasitaemia than those using mefloquine (odds ratio = 8.7, 7.4, and 4.1, respectively; p-level = 0.000001). In the multivariate model, other significant risk factors for malaria at delivery were first pregnancy, enrollment in the rainy or post-rainy season, maternal age of at most 25 years old, and seropositive to HIV.

The most important determinant of Plasmodium falciparum malaria infection in pregnant women was use of an effective intervention (i.e., chloroquine in an area with chloroquine-resistant parasites). Based on these findings, the researchers recommend that antimalarial programs focus on highly efficacious drugs and targeting pregnant women during the season of high malaria exposure. In sub-Saharan Africa, women frequently report of symptoms during pregnancy, some of which indicate possible illness. Given the adverse impact of malaria infection in pregnancy, these events may be important for at least two reasons: it may be possible to use reported fever illness as a determinant of which women need an antimalarial intervention, and, it is possible that adverse symptoms following the antimalarial intervention may be important determinants of continue adherence to the prevention regimen. In a cohort of pregnant women enrolled at first clinic visit in rural Malawi, Steketee et al. (23) evaluated reported fever, determined parasitaemia, and placed women on antimalarial regimens containing chloroquine or mefloquine.

A total of 4,187 pregnant women with parasitaemia attending 4 prenatal care clinics in rural Mangochi District, Malawi, were assigned to 1 of 4 regimens of antimalarial treatment and/or prophylaxis and followed through delivery. The regimens were 3 regimens for chloroquine, 1 of which was the current standard of care in Malawi, and a mefloquine regimen. Blood smear tests revealed the parasitaemia prevalence rate at enrollment to be 44.4%. The sensitivity of fever to identify parasitaemic pregnant women was 244%. Fever's specificity was 71%. Only high density parasitaemia (10,000 parasites per square meter) was associated with fever (44.9% versus 25.4% for no parasitaemia; odds ratio = 2.54; p = 0.001). The sensitivity of first or second pregnancy to identify parasitaemic women was 71%. Its specificity was 57%.

About 60% of women from both chloroquine and mefloquine treatment groups had side effects after a treatment dose. About 25% had side effects after a prophylactic dose. There were few serious effects. Among all women, the spontaneous abortion rate was 1.2% and the stillbirth rate was 3.9%. Women in the chloroquine and mefloquine treatment groups have similar abortion and stillbirth rates. Based on these findings, the

researchers concluded that using fever as a means to identify parasitaemic women is unreliable. They recommend antimalarial treatment and/or prophylaxis for all pregnant women, but when resources are limited it should be administered to women in their first or second pregnancy. A randomized, double-blind, placebo-controlled trial, which compared the effect of three interventions (weekly chloroquine prophylaxis, daily iron and weekly folic-acid supplementation, and case management of malaria) on congenital malaria, maternal hemoglobin and fetal outcome, was conducted among primigravidae resident in Hoima district, Uganda (24). Among 473 neonates examined at birth or within 7 days of life, 198 (42%) were parasitaemic, the level of parasitaemia in an infant being strongly correlated with those of placental ($p < 0.01$) and maternal, peripheral parasitaemia ($p < 0.01$). However, 33 (17%) of the parasitaemic neonates were born to mothers who had placental parasitaemia. Overall, 163 neonates were each examined for malarial parasites at birth and 1 month later. Of the 76 (47%) neonates found to have parasitaemia at birth, 37 (23%) appeared aparasitaemic at 1-month follow-up but 28 (17%) were still parasitaemic at that time.

Among the neonates born to the mothers who only received case management of malaria during pregnancy, parasitaemia at birth was associated with infant anemia at birth (i.e. < 140 grams hemoglobin per liter; $p = 0.03$). Infants found to be parasitaemic at 1-month follow-up had lower mean concentrations of hemoglobin at that time than their aparasitaemic counterparts ($p = 0.03$). Parasitaemia at birth was not significantly associated with low-birth-weight, in any of three intervention groups. The intervention given to the mother had no significant effect on the parasitaemia of her neonate, either at birth or at the age of 1 month. Congenital malaria per se may have little influence on birth-weight but may have an impact on infant anemia. In conclusion, congenital parasitaemia was not associated with birth-weight, but was related to anemia at birth in infants born to women who had only received active case management during their pregnancies. A cohort of 169 births to women who were exposed throughout pregnancy to chloroquine base 300 mg once a week for chemosuppression of malaria was studied (25). The birth defects in this cohort were compared with those in a control group of 457 births to women who were not exposed to chloroquine, most of whom lived in non-malarial areas.

The proportion of birth defects in the exposed group was not significantly different from that in the control group. This observation must be considered within the limitations of the study, which could detect only a strong teratogenic effect. It could not exclude risks lower than a 5.7-fold increase in the incidence of birth defects when chloroquine was used. Women using chloroquine during pregnancy for chemosuppression of malaria can be reassured that it is not a strong teratogen, but if it is to be used the risk of developing malaria should be balanced against the lack of data to determine whether it carries a low teratogenic risk.

Chloroquine, its N-Dealkylated Metabolites, and chloroquine N-oxides were detected in the urine of pregnant women who received chloroquine medication whereas chloroquine and its nonpolar metabolites, desethyl- and didesethylchloroquine and 7-chloro-4-aminoquinoline, have been found in the neonates' urine, blood, and cord blood (26). Chloroquine and its relatively non-polar metabolites (including one without the alkyl side-chain, 7-chloro-4-aminoquinoline) cross the placenta is demonstrated by the presence of these

compounds in the cord blood, neonatal systemic blood, and neonatal urine. In malaria-endemic regions, the impact of malaria upon pregnancy and the value of chemoprophylaxis for malaria for pregnant women remain controversial. Nyirjesy et al. (27) prospectively studied 302 pregnant women who presented in labor to Centre Medical Evangelique, Nyankunde, Zaire. They evaluated the incidence of malarial infection mothers, placenta, and neonates and examined the effect of infection on birth weight and perinatal mortality rate. These authors analyzed the outcome of pregnancy in relation to prophylaxis with chloroquine, controlling for parity and prenatal clinic attendance.

Peripartum smears of maternal blood (21%), placentas (33%), cord blood (9%), and neonatal blood (7%) were positive for Plasmodium falciparum. Maternal malaria increased the risk of perinatal death (relative risk = 12.4) and low-birth-weight (relative risk = 3.7). Neonatal malaria increased the risk of perinatal death (relative risk = 7.2). Chloroquine prophylaxis protected against maternal (relative risk = 0.4) and fetal malaria (relative risk = 0.2), low-birth-weight (relative risk = 0.39), perinatal death (relative risk = 0.38). Peripartum malaria increases the risk of perinatal death and low-birth-weight. Chemoprophylaxis with chloroquine during pregnancy may have a protective effect, even in certain areas where chloroquine-resistant Plasmodium falciparum is endemic.

Characteristics of women in the chloroquine group were similar to those in the intermittent preventive treatment in pregnancy group. Briand et al. (28) shoved that women in the intermittent preventive treatment in pregnancy group had a significantly decreased risk of delivering a neonate with a low-birth-weight (adjusted odds ratio = 0.54; 95% confidence interval = 0.38-0.78), and placental infection (adjusted odds ratio = 0.15; 95% confidence interval = 0.09-0.24). The evidences that intermittent preventive treatment in pregnancy is substantially more beneficial than the chloroquine group for prevention of malaria during pregnancy. In areas of stable transmission, malaria during pregnancy is associated with severe maternal and fetal outcomes, especially low-birth-weight. To prevent these complications, weekly chloroquine chemoprophylaxis is now being replaced by intermittent preventive treatment with sulfadoxine-pyrimethamine in West Africa (29). The prevalence of placental malaria and its burden on low-birth-weight were assessed in Benin to evaluate the efficacy of weekly chloroquine chemoprophylaxis, prior to its replacement by intermittent preventive treatment. Among 1,090 singleton live births, prevalence of placental malaria and low-birth-weight were 16% and 17%, respectively.

After adjustment, there was a non-significant association between placental malaria and low-birth-weight (adjusted odds ratio = 1.43; $p = 0.10$). Multiple linear regressions showed a positive association between placental malaria and decreased birth weight in primigravidae. More than 98% of the women reported regular chemoprophylaxis and chloroquine was detectable in 99% of urine samples. Protection from low-birth-weight was high in women reporting regular chloroquine prophylaxis, with a strong duration-effect relationship (test for linear trend, $p < 0.001$). Despite high parasite resistance and limited effect on placental malaria, a chloroquine chemoprophylaxis taken at adequate doses showed to be still effective in reducing low-birth-weight in Benin.

3-7. Chemoprophylaxis of malaria with chloroquine in children

In the Gambia, 760 children aged less than 10 years with Plasmodium falciparum malaria infection were treated with chloroquine (25 mg/kg) and followed-up 2 and 9 days after the start of treatment (30).

A total of 700 children (92.1%) completed the study. The level of in-vivo chloroquine resistance was varied by areas, from 0.4% to 16%. Of the 28 children found to have chloroquine resistant malaria, none was ill when seen at 9 days follow-up and only 3 (10.3%) required further treatment with alternative antimalarial drugs because of persistent high levels of parasitaemia. However, the fact that 30.4% of the children who completed the study had chloroquine in their urine at presentation may have masked the true level of resistance and led to underestimation of the clinical significance of these findings. The blood film at day 2 did not usefully predict resistance. Sixty-seven isolates were tested in-vitro for chloroquine sensitivity. The mean half maximal effective concentration (EC_{50}) was 15.5nmol/l an eight-fold decrease in sensitivity from that of isolates tested in 1982. Eight (11%) of the isolates had EC_{50}s above the WHO reference value for sensitive isolates of 18.3 nmol/l, with values ranging from 22 to 65 nmol/l of culture medium.

Gambian isolates were sensitive to quinine (mean EC_{50} = 49.6 nmol/l). A case-control study was conducted to evaluate the efficacy of the combination of chloroquine plus proguanil as malaria prophylaxis in a non-immune population living in the Central African Republic (31). Cases were patients presenting with a malaria attack confirmed by positive blood film and/or a histidine-rich protein 2 (HRP2) positive antigen test at the Pasteur Institute of Bangui. Two control subjects were included per case: one was a relative or close friend and the other was matched to the patient with respect to the length of stay. A questionnaire assessing malaria prophylaxis habits and malarial risk factors over the 2-month period prior to inclusion in the study was given to 48 cases and 96 controls. A conditional logistic regression was used to identify risk factors. The efficacy of chloroquine plus proguanil regimen was found to be high (95.5%; 95% confidence interval = 74.0-99.2%) in this country known for high chloroquine resistance. These present findings lend some support to the use of chloroquine plus proguanil in Bangui, and the protective efficacy of chloroquine plus proguanil should now be studied prospectively as part of a randomized controlled trial of various prophylactic drugs. One hundred and ninety-eight Nigerian children who received weekly chemoprophylaxis with chloroquine from shortly after birth until the age of one year or two years and 185 age-matched controls were studied (32).

Chemoprophylaxis with chloroquine was partially, but not completely, effective in controlling malaria infection. Clinical malaria was documented significantly less frequently in protected children than in control children, and only 9% of random blood films obtained from protected children were positive for Plasmodium falciparum while 41% of random blood films from control children were positive for this parasite. Mean malaria antibody levels were lower in protected than in control children; for ELISA and precipitin antibodies the difference between the two groups was less marked at two years than at one year. Mortality rate was similar among protected and among control children. No rebound mortality or morbidity was observed after chemoprophylaxis was stopped.

A clinical trial of malaria prophylaxis using a single dose of chloroquine at different intervals was carried out to determine if chloroquine could be given at intervals longer than advocated (33). The usual adult prophylaxis of chloroquine recommended was 300 mg base once a week or 600 mg base once in 2 weeks, the dose being proportionately less for children. A regrouped village consisting of 5 small villages in Chief Chikuwe's area

(Zambia) was selected. The closest rural health center was situated 9 kilometers away from this village so the villagers did not have easy access to chloroquine tablets. The total population of the regrouped village with the exception of the primary-school-children (who received regular chloroquine prophylaxis at their schools once a month) were placed in 4 groups A, B, C, and D using the random number tables. Each group had about 55-59 participants. On the 1st visit, blood samples were collected from everybody and thick and thin films were made from each individual's samples.

All members of groups B, C, and D were given chloroquine, corresponding to his/her age. Chloroquine phosphate was used and the dose administered was as follows: 15 years and older, 600 mg base; 10-14 years, 300 mg base; 5-9 years, 150 mg base; and 0-4 years, 75 mg chloroquine base. Individuals belonging to group A were given placebo. The 2nd visit was made 1 month after the 1st and at this chloroquine was given to group B only. All the other groups received the placebo. On the 3rd visit, 2 months after the 1st visit in, groups B and C received the chloroquine and groups A and D received placebo. During the 4th visit, 3 months after the 1st visit, groups B and D received the chloroquine and groups A and C received placebo. Out of a total of 230 participants examined, 47 were found to be positive for malarial parasites giving a parasite rate of 20.43%. This parasite rate was found to be evenly distributed among the 4 study groups. The age group 12 months-14 years had the highest prevalence, the parasites rate being 32.26%. In group A, the control group, there was hardly any change in parasite rate. In group B, who received the prophylactic once in one a month, the parasite rate fell from 18.97% to 1.79% at end of the study period. In group D, who received the prophylactic once 3 months, the parasite rate fell from 24.14% to 8.77% at the end of 3 months. The present survey conducted between December 1997 and August 1998 at the Chantal Biya Maternity Section of the Ebolowa Provincial Hospital, Cameroon (34). A total of 231 parturient who give birth to 232 neonates were included the study. Ninety-five of them (41.1%) took antimalarial prophylaxis with chloroquine in the indexed pregnancy, and 136 (58.9%) did not. Both groups were similar with respect to socio-demographic characteristics except for educational level of the mother, which was significantly higher in the group of prophylaxis (Chi-square = 8.5; p = 0.02). The overall prevalence of maternal parasitaemia was 37.2%. The group on chloroquine experienced a lesser parasitaemia (26.3%) than the non-prophylactic group (44.9%; odds ratio = 2.28; 95% confidence interval = 1.24-4.19). The proportion of women with severe parasitaemia (> 4,000 parasites per μl) was also lower in the chloroquine group than in non-prophylactic group (17.6% versus 7.3%; odds ratio = 2.86; 95% confidence interval = 1.04-7.23).

A modest reduction in low-birth-weight was found in the neonates born to mothers treated with chloroquine which was not significant (23.4% versus 16.0%; p = 0.16). In conclusion, chloroquine given to prevent malaria in pregnancy was found to be effective in reducing peripheral malaria parasitaemia, but improvement in birth-weight could be demonstrated. Among other factors, impaired biological activity of the drug at the level of the placenta where parasite sequestration frequently occurs might be the explanation. Salihu et al. (34) recommend that further investigation be carried out in the study area to evaluate this finding, and if confirmed, institute appropriate changes in the present policy of chloroquine prophylaxis in pregnancy.

3-8. Resistance of Plasmodium parasites to chloroquine in children

Over 12 years, from 1984 to 1995, Trape et al. (35) conducted a prospective study of overall and malaria specific mortality among three rural populations in the Sahel, savanna and forest areas of Senegal. The emergence of chloroquine resistance has been associated with a dramatic increase in malarial mortality rate in each of the studied populations. After the emergence of chloroquine resistance, the risk of malaria death among children 0 to 9 years old in the three populations was multiplied by 2.1, 2.5, and 5.5, respectively. In this study documents malaria mortality rate at the community level in Africa before and after the emergence of chloroquine resistance. These findings suggest that the spread of chloroquine resistance has had a dramatic impact on the level of malaria mortality in most epidemiological context in tropical Africa.

Response of Plasmodium falciparum to chloroquine treatment was assessed in-vivo in 219 malaria cases from eight villages in a formerly hypoendemic area of Zimbabwe experiencing a malaria outbreak (36). Seven (3%) of the cases were fully sensitive to chloroquine while 182 (83%) exhibited chloroquine-resistant responses. Of the 182 chloroquine-resistant cases 74 (41%) showed resistance index (RI) resistance while 108 (59%) exhibited RII-RIII resistance. In-vivo follow-up was not completed, to day 28 in the remaining 30 (14%) of the malaria cases, which were therefore either fully sensitive or RI resistant. In 23 (11%) of the malaria cases pyrexia and increasing parasitaemia occurred between day 3 and day 7 after treatment. Mean parasite clearance time was 5.8 days$\pm$2.89 days in patients who were cleared of asexual parasitaemia. In all but 1 (0.5%) of the chloroquine-resistant infections, asexual parasites were cleared on day 7 following treatment with the sulphadoxine-pyrimethamine combination (Fansidar). These present findings show an acute problem of chloroquine resistance in an area of Zimbabwe. It is recommended that the drug policy be modified to allow distribution of limited stocks of Fansidar to the local clinics for restricted use on documented chloroquine treatment failure within 7 days. Zambian children under-five years with malaria infection were selected on a positive thick blood smear for Plasmodium falciparum of at least 1,000 parasites per μl on the day of admission (37). During a chloroquine course, daily follow-up of parasitaemia was performed. Blood samples were taken on day 0 and day 3 to measure chloroquine levels before admission and after treatment. Eighty-four patients were evaluated. Forty-eight patients did not meet criteria. Thirty-six patients met all criteria, of which 16 (44.5%) patients were infected with sensitive strains and 20 (55.5%) with resistant strains of Plasmodium falciparum.

In West Africa, administration of chloroquine chemoprophylaxis during pregnancy is common, but little is known about this impact on Plasmodium falciparum infection during pregnancy (38). Therefore, cross-sectional studies in antenatal care clinics and delivery units were conducted in Koupela District, Burkina Faso. Chloroquine chemoprophylaxis was reported by 69% of 597 pregnant women at antenatal care clinics and by 93% of 853 women in delivery units. Plasmodium falciparum peripheral parasitaemia was identified in 29% of women at both antenatal care clinics delivery units. Placental parasitaemia was identified in 22% of delivering women and was strongly associated with low-birth-weight (risk ratio = 2.9; 95% confidence interval = 1.6-5.4). In multivariate analysis, use of chemoprophylaxis was not associated with a reduction in the prevalence of placental

parasitaemia, low-birth-weight, or prematurity. Despite the high reported chloroquine chemoprophylaxis coverage, peripheral and placental malaria rates remain high and are associated with known adverse outcomes during pregnancy, including maternal anemia, prematurity, and low-birth-weight. Alternative prevention strategies, such as use of insecticide-treated mosquito bed-nets and intermittent preventive treatment with more-effective antimalarials, are needed. Although chloroquine monotherapy is now generally inadequate for the treatment of Plasmodium falciparum malaria in northern Ghana, 58% of 225 children failed treatment by day 14 use of the drug continues because of its low cost and wide availability (39). The risk factors associated with chloroquine-treatment failure in this region of Africa, including the T76 mutation in the chloroquine resistance transporter (pfcrt) gene and the Y86 mutation in the multidrug resistance (pfmdr1) gene of Plasmodium falciparum, have now been investigated, and genotyped-failure indices have now been calculated. Treatment failure was found to be associated with young age, poor nutritional status, pfcrt T76 and pfmdr1 Y86, and early treatment failure was also associated with high parasitaemia.

The presence and concentration of 'residual' chloroquine in the blood of patients immediately before they were treated with chloroquine for the study appeared to have no effect on outcome. Presence at recruitment of pfcrt T76 or pfmdr1 Y86 or both mutations increased the risk of treatment failure by 3.2-, 2.4- and 4.5-fold, and the risk of early treatment failure by 9-8-, 2.7-and 10.2-fold, respectively. These indices were relatively low in the younger children, those with malnutrition, and those with high parasitaemia when treated. Residual chloroquine did not affect the genotype-failure indices substantially. Both pfcrt T76 genotype-failure indices for clinical and all treatment failures were 2.8 and 1.4, respectively. These indices were relatively low in the younger children, those with malnutrition, and those with high parasitaemia when treated. Residual chloroquine did not affect the genotype-failure indices substantially. Both pfcrt T76 and, to a lesser extent, pfmdr1 Y86, would be useful tools for the surveillance of chloroquine resistance in northern Ghana. In the current transition phase to alternative first-line treatment for Plasmodium falciparum malaria, it should be possible to provide estimates of the level of chloroquine resistance by monitoring the prevalence of these mutations. The study included 45 cases of neonatal malaria. Thirty cases of malaria, admitted during first days of life, diagnosed as congenital malaria, were kept in group A, while 15 cases admitted in the ward from the age of 11 to 28 days, labeled as acquired malaria, were named group B (40). The diagnosed cases were treated with the standard dose of chloroquine sulphate. Those patients who improved clinically as well as revealed no parasite on follow-up were labeled as chloroquine sensitive.

On the other hand, children show poor clinical response with persistence of the parasites in the blood or initially disappearing but later again having a clinical disease with malaria parasite on follow-up, were labeled as chloroquine resistant. They were treated with quinine sulphate. Outcome was compared in both groups regarding the pattern of chloroquine resistance and death/survival. Data was collected on which Fischer' exact test of significance was performed to know the level of significance. P-level of < 0.05 was taken as statistically significant. Low-birth-weight, severe hemolytic anemia with history of fever in the mother were main features in group A while in group B fever, anemia and history of blood

transfusion were marked features. In group A 76.66% were caused by Plasmodium falciparum. While in group B 60% were caused by Plasmodium vivax. In group A 26.66% were chloroquine resistant while 33.65% were chloroquine resistant in group B. The mortality rate was 16.66% in group A and 13.33% in group B. Intrauterine growth retardation, hemolytic jaundice and history of fever in the mother in the last trimester of pregnancy in the congenital fever, history of blood transfusion in neonates in acquired malaria but pallor in both groups, were important features. Pattern of chloroquine resistance and mortality rate in both groups was not statistically different.

Malaria remains a significant health problem in Yemen with Plasmodium falciparum being the predominant species which is responsible for 90% of the malaria cases. Despite serious concerns regarding increasing drug resistance, chloroquine is still used for the prevention and treatment of malaria in Yemen. Al-Mekhlafi et al. (41) determined the prevalence of chloroquine resistance of Plasmodium falciparum isolated from Yemen based on the pfcrt T76 mutation. A cross-sectional study was carried out among 511 participants from four governorates in Yemen. The prevalence of pfcrt T76 mutation was 81.5% (66 to 81 isolates). Coastal area/foothills had higher prevalence of pfcrt T76 mutation compared to highland areas (90.5% versus 71.8%; p = 0.031).

The pfcrt T76 mutation had a significant association with parasitaemia (p = 0.045). Univariate analysis showed a significant association of pfcrt T76 mutation with people aged > 10 years (odds ratio = 5; 95% confidence interval = 2.3-36.2; p = 0.001), low household income (odds ratio = 5; 95% confidence interval = 1.3-19.1; p = 0.027), no insecticide spray (odds ratio = 3.7; 95 confidence interval = 1.16-11.86; p=0.025), and not sleeping under insecticide treated bed-nets (odds ratio = 4.8; 95% confidence interval = 1.38-16.76; p = 0.01). Logistic regression model confirmed age > 10 years and low household income as predictors of pfcrt T76 mutation in Yemen Plasmodium falciparum isolates. The high prevalence of pfcrt T76 mutation in Yemen could be a predictive marker for the prevalence of Plasmodium falciparum chloroquine resistance. The present findings show the necessity for an-vivo therapeutic efficacy test for chloroquine. Plasmodium falciparum chloroquine resistance should be addressed in the national strategy to control malaria infection.

Tinto et al. (42) investigated the relationship between the two main molecular markers for chloroquine resistance (pfcrt T76 and pfmdr1 Y86) and the clinical efficacy of amodiaquine in Burkina Faso. Before treatment, the prevalence of pfcrt T76, pfmdr1 Y86 or both mutations in the same infection was significantly higher in patients who experienced a recrudescence than in those who successfully responded to the treatment. Therefore, these two molecular markers could be useful in monitoring amodiaquine resistance, particularly in countries where this drug is used in combination with artesunate as first- or second-line treatment.

Plasmodium falciparum resistance to antimalarial drugs remains a major obstacle to the control of malaria (43). In 2001, Tanzania replaced chloroquine with sulphadoxine-pyrimethanine and in 2006 sulphadoxine-pyrimethanine was replaced with artemisinin combination therapy. Seven hundred and forty one samples were genotyped. The current frequency of the chloroquine-susceptible pfcrt K76 was above 92% and did not differ between regions in Tanzania (Chi-square = 2.37; p = 0.795). The K76 allelic prevalence was between 85.7% and 93% in regions (Chi-square = 7.88; p = 0.163). The chloroquine

resistant recovery trends showed regional variability that may be caused by differences in malarial transmission intensity, but overall the trends converge as the susceptibility levels in all regions approached > 90%. Chloroquine withdrawal in Tanzania has resulted into > 90% recovery of susceptibility in ten years of withdrawal. These findings are in support of the search for chloroquine-base combination drugs as a possible future alternative to sulphadoxine-pyrimethanine for intermittent preventive therapy in pregnancy in places where full recovery of chloroquine-susceptibility will be evident. In Malawi, cessation of chloroquine use was followed by the re-emergence of chloroquine-susceptible malaria. This observation suggests that there is continuing use of ineffective anti-malarials in Africa and that persistent chloroquine-resistant malaria is due to ongoing drug pressure despite national policy changes (44). Chloroquine was the most common anti-malarial used according to surveys from 14 of 21 countries analyzed, predominantly in West Africa.

Among eight countries with longitudinal molecular resistance data, the four countries where the highest proportion of children treated for fever received chloroquine (Uganda, Burkina Faso, Guinea Bissau, and Mali) also showed no significant declines in the prevalence of chloroquine-resistant infections. The three countries with low or decreasing chloroquine use among children who reported fever treatment (Malawi, Kenya, and Tanzania) had statistically significant declines in the prevalence of chloroquine resistance. The present findings demonstrate that in 2006-2007, chloroquine continued to be used at high rates in many African countries. In countries reporting sustained chloroquine use, chloroquine-resistant malaria persists. In contrast, a low level of estimate chloroquine use is associated with a declining prevalence of chloroquine resistance. In literature, there are no data on the metabolism and pharmacokinetics of chloroquine in neonates, infants, and children.

4- DISCUSSION

Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi are the malaria parasites that infect humans. Plasmodium falciparum and Plasmodium vivax cause most of the malarial infections worldwide. Plasmodium falciparum accounts for the majority of malaria infection in sub-Sahara Africa and is associated with most severe disease. Plasmodium vivax accounts for half of malaria infection in South and East Asia and > 80% of the malarial infections in the America (2). Chloroquine was, for a long time, the world's most widely used antimalarial drug, but the most common and virulent parasite, Plasmodium falciparum, Plasmodium vivax, and Plasmodium ovale are now increasingly resistance. Chloroquine-sensitive Plasmodium falciparum parasite concentrates chloroquine to higher levels than did chloroquine-resistant organisms (45). Chloroquine is well absorbed, widely distributed in body tissues, slowly metabolized by the liver and very slowly cleared from the body.

Residents in malaria endemic areas develop considerably immunity over time, but pregnancy makes women more vulnerable, and infection during pregnancy increases the risk of anemia, miscarriage, stillbirth, and prematurity (1). Aderounm et al. (7) observed that a chloroquine dose of 25 mg/kg eliminated the asexual forms of Plasmodium falciparum from the blood of children after 3 days of treatment and the blood remained negative for the rest of 7 days of treatment. The case-fatality rate was 13% in children treated with chloroquine and case-fatality was 4.1% in

children treated with a regimen that would eliminate and clear parasitaemia. The proportion of deaths attributable to chloroquine was 69% (10). Kebede et al. (11) assessed the in-vivo treatment efficacy of chloroquine to Plasmodium falciparum malaria infection using the standard WHO 14- day treatment response monitoring guideline in 427 patients. Tendency to remain febrile on subsequent follow-up days was also more observed in children than adults. Neely et al. (12) conducted a pilot study to determine if chloroquine can reduce HIV vertical transmission in pregnant women. Such random maternal chloroquine use may be associated with a decrease rate of HIV vertical transmission. A three-day treatment with 25mg/kg chloroquine was performed in Afghan refugees in Pakistan and extends five-day treatment of 40 mg/kg for recrudescent infection, based on the assumption that five-day courses would more likely achieve a cure (13).

Fever clearance time, parasite clearance time, and proportions gametocytaemic post-treatment were similar between treatment groups. A percentage of 84 patients treated with 25 mg/kg chloroquine versus 51% of patients treated with 40 mg/kg chloroquine experienced parasite recrudescence during follow-up. Cure rates were significantly improved with 40 mg/kg chloroquine. Chloroquine-resistance marker 76T was found in all isolates analyzed, while pmdr1 86Y and 184Y were found in 18% and 37% of isolates, respectively. Chloroquine is not suitable for first-line Plasmodium falciparum treatment in Afghan refugee community. Nsimba and Rimony (14) identified treatment strategies adopted by 500 mothers or guardians of sick children under 5 years who visited the public facilities seeking care. Eight-five per cent of the sick children given chloroquine had whole blood chloroquine levels above 500nmol/l and 33% of the sick children with whole blood chloroquine above 1,000nmol/l had malaria parasites in their blood. Chloroquine resistance requires a switch to another antimalarial drug. Sulfadoxine-pyrimethamine is used after chloroquine treatment failure. Kamya et al. (15) compared sulfadoxine-pyrimethamine treatment with chloroquine treatment. The clinical failure was 54% after treatment with chloroquine and 11% after treatment with sulfadoxine-pyrimethamine treatment ($p < 0.001$). Although early parasite clearance was significantly better in the sulfadoxine-pyrimethamine group ($p = 0.001$), fever clearance at day 3 was the same with the two antimalarial drugs.

Resistance of Plasmodium falciparum to chloroquine is widespread in Papua New Guinea. In 1997, it was decided to replace chloroquine with amodiaquine in combination with sulfadoxine-pyrimethamine (16). A total of 418 children enrolled in the study 95.5% had an adequate clinical response to the new therapy. Out of the 19 children who did not have an adequate response, 3 (0.7% of the total) developed severe signs in the first 24 hours. From these results it was concluded to introduce the two-drug combination regimen as the standard first-line treatment of uncomplicated malaria in Papua New Guinea. A total of 250 pregnant Nigerian women were assigned to receive either sulfadoxine-pyrimethamine given twice or presumptive chloroquine treatment followed by weekly pyrimethamine (17). Four (1.8%) of pregnant women receiving sulfadoxine-pyrimethamine and 22 (9.8%) of the pregnant women treated with chloroquine plus pyrimethamine had a febrile illness ($p = 0.005$). None in the sulfadoxine-pyrimethamine group but 11(4.9%) pregnant women in the chloroquine plus pyrimethamine group had peripheral parasitaemia prior or during delivery. Intermittent preventive treatment with sulphadoxine-pyrimethamine is superior to

chloroquine plus pyrimethamine for prevention of malaria infection and anemia in Nigerian pregnant women. A randomized comparison of the in-vivo efficacy of chloroquine alone, sulfadoxine-pyrimethamine alone, and their combined administration was carried out in the third quarter of 2001 in Kaberamaido District, northeastern Uganda [18]. In terms of clinical cure rate, speed of clinical relief and parasite clearance the combined treatment proved to be the most effective of the three drug regimens. Legros et al. (19) assessed the efficacy of chloroquine and sulfadoxine-pyrimethamine for treating uncomplicated Plasmodium falciparum malaria infection in Uganda. The treatment failure rates were 81.1% of children treated with chloroquine and 25.0% of the children treated with sulfadoxine-pyrimethamine. The efficacy of treatment is better with sulfadoxine-pyrimethamine than with chloroquine.

Malaria in pregnancy is one of the most common preventable causes of maternal and neonatal morbidity and mortality rate in sub-Saharan Africa. To prevent its adverse effects, such as maternal anemia, placental parasitaemia and low-birth-neonates, the WHO recommends effective malaria case management, use of insecticide bed-nets and intermittent preventive therapy in pregnancy. Sulfadoxine-pyrimethamine has been the standard for intermittent preventive in pregnancy in several countries, but parasite resistance to sulfadoxine-pyrimethamine is growing and new intermittent preventive therapies in pregnancy are urgently needed. Chandra et al. (20) suggest the use of azithromycin plus chloroquine intermittent preventive therapy in pregnancy. The WHO recommends intermittent preventive treatment with sulfadoxine-pyrimethamine in Africa. Kimani et al. (21) studied the efficacy, tolerability, and safety of a fixed-dose of 250 mg azithromycin plus 155 mg chloroquine base for intermittent preventive therapy in pregnancy relative to the intermittent preventive therapy in pregnancy with sulfadoxine-pyrimethamine. There was no significant difference in the incidence of low-birth-weight between treatments with the two regimens. Intermittent preventive therapy in pregnancy of azithromycin plus chloroquine was less well-tolerated in mothers than intermittent preventive therapy in pregnancy of sulfadoxine-pyrimethamine. Congenital anomalies, deaths, and serious adverse events were comparable in neonates for both groups. Steketee et al. (22) examined three regimens using chloroquine and one regimen using mefloquine. The efficacy of the regimens was determined by comparing rates of clearance of initial parasitaemia; prevention of breakthrough infection and parasitaemia at delivery in maternal peripheral blood, placental blood, and in infant umbilical cord blood. Compared with women on mefloquine, women on a chloroquine regimen were at significantly greater risk of persistent and breakthrough infection. At delivery, women on a chloroquine regimen faced a significantly higher risk of peripheral, placental, or umbilical cord parasitaemia than those using mefloquine.

In sub-Saharan Africa, women frequently report of symptoms during pregnancy, some of which indicate possibly illness. Fever illness needs an antimalarial intervention. In a cohort of pregnant women enrolled at first clinic visit in rural Malawi, Steketee et al. (23) evaluated reported fever, determined parasitaemia, and placed women on antimalarial regimens containing chloroquine or mefloquine. Blood smear tests revealed the prevalence rate at enrollment to be 44.4%. The sensitivity of fever to identify parasitaemic pregnant was 244%. Fever's specificity was 71%. Only high density parasitaemia (10.000 parasites per square

meter) was associated with fever (44.9% versus 25.4% for no parasitaemia; p = 0.001). About 60% of women from both chloroquine and mefloquine treatment groups had side effect after a treatment dose. Women in the chloroquine and mefloquine treatment groups had similar abortion and stillbirth rates. The researchers recommend antimalarial treatment and/or prophylaxis for all pregnant women. Among 473 neonates examined at birth or 7 days of life, 42% were parasitaemic (24). Overall, 163 neonates were examined for malaria parasites at birth and 1 month later. A percentage of 47 neonates found to have parasitaemia at birth, 23% appeared aparasitaemic at 1-month follow-up, but 17% were still parasitaemic at that time. Among the neonates born to mothers who only received case management of malaria during pregnancy, parasitaemia at birth was associated with infant anemia at birth. Parasitaemia at birth was not significantly associated with low-birth-weight. Congenital malaria per se may have little influence on birth-weight, but may have an impact on infant anemia.

A cohort of 169 births to women who were exposed throughout pregnancy to 300 mg chloroquine once a week had birth defects compared with those in a control group of 457 births to women who were not exposed to chloroquine (25). The women whom using chloroquine during pregnancy for chemosuppression of malaria can be reassured that it is not a strong teratogen. Nyirjesy et al. (27) analyzed the outcome of pregnancy in relation to prophylaxis with chloroquine in 302 pregnant women. Peripartum smears of maternal blood (21%), placentas (33%), cord blood (9%), and neonatal blood (7%) were positive for Plasmodium falciparum. Maternal malaria increased the risk of perinatal death and low-birth-weight. Neonatal malaria infection increases the risk of perinatal death. Chloroquine prophylaxis protected against maternal and fetal malaria, low-birth-weight, and perinatal death. Peripartum malaria increases the risk of perinatal death and low-birth-weight. Chemoprophylaxis with chloroquine during pregnancy may have a protective effect, even in areas where chloroquine-resistant Plasmodium falciparumis endemic. In areas of stable transmission, malaria during pregnancy is associated with severe maternal and fetal outcomes, especially low-birth-weight. To prevent these complications, weekly chloroquine chemoprophylaxis is now being replaced by intermittent preventive treatment with sulfadoxine-pyrimethamine in West Africa (29). Multiple linear regressions showed a positive association between placental malaria and decreased birth-weight in primigravidae. Protection from low-birth-weight was high in women reporting regular chloroquine prophylaxis, with a strong duration-effect relationship.

In Gambian, 760 children aged less than 10 years with Plasmodium falciparum malaria infection were treated with 25 mg/kg chloroquine and followed-up 2 and 9 days after the start of treatment (30). Of 28 children found to have chloroquine-resistant malaria, none was ill when seen at 9 days follow-up and 3 (10.3%) required further treatment with alternative antimalarial drugs because of persistent high levels of parasitaemia. A case-control study was conducted to evaluate the efficacy of the combination of chloroquine plus proguanil as malaria prophylaxis in a non-immune population living in the Central African Republic (31). There were 48 cases and 96 controls. The efficacy of chloroquine plus Proguanil regimen was found to be high (95.5%). The present findings lend some support to the use of chloroquine plus proguanil in Bangui. A total of 195 Nigerian children who received weekly chemoprophylaxis with chloroquine from shortly after birth until the age of one year or two years and 185

age-matched controls were studied (32). Chemoprophylaxis with chloroquine was partially, but not completing, effective in controlling malaria infection. Clinical malaria was documented significantly less frequently in protected children than in control children. Mean malaria antibody levels were lower in protected than in control children. Karunakaran (33) observed that, in school-aged-children, the treatment with chloroquine-phosphate reduced the malarial parasites compared with children who received placebo.

A total of 231 parturient women who give birth to 232 neonates were enrolled. Ninety-five (41.1%) took antimalarial prophylaxis with chloroquine, and 136 (58.8%) did not (34). The group on chloroquine experienced a lesser parasitaemia (26.3%) than the non-prophylactic women (44.9%). The proportion of women with severe parasitaemia (> 4,000 parasites per μl) was also lower in the chloroquine group than in non-prophylactic women (17.6% versus 7.3%). Chloroquine given to prevent malaria in pregnancy was found to be effective in reducing peripheral malaria parasitaemia. In West Africa, treatment for the prevention of malaria during pregnancy was changed for chloroquine prophylaxis to intermittent preventive treatment (28). The women in the intermittent preventive treatment group had a significantly decreased risk of delivering infants with low-birth-weight.

Over 12 years, from 1984 to 1995, Trape et al. (35) conducted a prospective study on malarial specific mortality rate among three rural populations in the Sahel, savanna and forest areas of Senegal. The emergence of chloroquine resistance was associated with a dramatic increase in mortality rate in each of the studied populations. The risk of malaria death among children 0 to 9 years old in the three populations studied was multiplied by 2.1%, 2.5% and 5.5%. These findings suggest the spread of chloroquine resistance had a dramatic impact on the level of malarial mortality in most epidemiological context in tropical Africa.

Response of Plasmodium falciparum to chloroquine treatment was assessed in-vivo in 219 malaria cases from eight villages in a formerly hypoendemic area of Zimbabwe experiencing a malaria outbreak (36)]. Seven (3%) of the cases were fully sensitive to chloroquine while 182 (83%) exhibited chloroquine-resistant responses. Mean parasite clearance time was 5.8±2.89 days in patients who were cleared of asexual parasites. The present findings show an acute problem of chloroquine resistance in an area of Zimbabwe. In Zambia, 84 children under-five years with malaria infection with positive thick blood smear for Plasmodium falciparum of at least 1,000 parasites per μl on the day of admission (37). During a chloroquine course, daily follow-up of parasitaemia was performed. A percentage of 44.5 children were infected with sensitive to chloroquine sensitive strains and 55% children were resistant strains of Plasmodium falciparum.

Chemoprophylaxis was reported by 69% of 597 pregnant women at antenatal care clinics and 93% of 853 women were treated in delivery units (38). Plasmodium falciparum peripheral parasitaemia was identified in 29% of women at both antenatal care clinics and delivery units. Placental parasitaemia was identified in 22% of delivering women and was associated with low-birth-weight. Chemoprophylaxis with chloroquine was not associated with a reduction in the prevalence of placental parasitaemia, low-birth-weight, or prematurity. Despite the high reported chloroquine chemoprophylaxis coverage, peripheral and placental malaria rates remaining high and are associated with adverse outcomes during pregnancy, including maternal anemia, prematurity, and low-birth-weight.

Chloroquine monotherapy is inadequate for the treatment of Plasmodium falciparum. In Ghana, 58% of 225 children failed treatment because continuous treatment with chloroquine (39). The risk factors associated with chloroquine-treatment failure in this region of Africa include the T76 mutation in the chloroquine resistance transporter (pfcrt) gene and the Y86 mutation in the multidrug resistance (pfmdr1) gene of Plasmodium falciparum.

Treatment failure was found to be associated with young age, poor nutrition status, pfcrt T76 and pfmdr1 Y86, and early treatment failure was also associated with high parasitaemia. Presence at recruitment of T76 or pfmdr1 Y86 or both mutations increased the risk of treatment failure by 3.2-, 2.4- and 4.5-fold, and the risk of early treatment failure by 9- 8- and 10.2-fold, respectively. Malaria remains a significant health problem with Plasmodium falciparum being the predominant species which is responsively for 90% of malarial cases. Al-Mekhlafi et al. (41) determined the prevalence of chloroquine resistance of Plasmodium falciparum isolated from Yemen base on the pfcrt T76 mutation. The pfcrt T76 mutation had significant association with parasitaemia ($p = 0.045$).

The high prevalence of pfcrt T76 mutation in Yemen could be a predictive marker for the prevalence of Plasmodium falciparum chloroquine resistance. The two main molecular markers for chloroquine resistance (pfcrt T76 and pfmdr1), and the clinically efficacy of amodiaquine in Burkina Faso were studied by Tinto et al. (42). Before treatment, the prevalence of pfcrt T76, pfmdr1 Y86 or both mutations was significantly higher in patients who experience a recrudescence than in those who successfully responded to the treatment. These two markers could be useful in monitoring amodiaquine resistance. Because of chloroquine resistance Tanzania replaced chloroquine with sulphadoxine-pyrimethanine in 2001; and in 2006 replaced sulphadoxine-pyrimethanine with artemisinin combination therapy (43). Chloroquine withdrawal in Tanzania resulted into > 90% recovery of susceptibility in ten years. A similar effect was observed Malawi (44). These findings suggest that there is continuing use of antimalarials in Africa and that persistent chloroquine is due to ongoing drug pressure despite national policy change.

5- CONCLUSION

In conclusion, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi are the plasmodium parasites that infect humans. Plasmodium falciparum and Plasmodium vivax cause most of the malarial infections worldwide. Plasmodium falciparum is the most common and virulent parasite. Chloroquine was for a long time, the world's most widely used antimalarial drug, but Plasmodium falciparum is now increasingly resistance. Chloroquine-sensitive Plasmodium falciparum concentrates chloroquine to higher levels than did chloroquine-resistant parasite. In 2010, there were an estimated 219 million cases of malaria infection resulting in 660,000 deaths. Two thirds of deaths occurred in children, children are more vulnerable than adults to malaria infection. In sub-Saharan Africa, maternal malaria infection is associated with up 200,000 estimated infants' deaths yearly. Chloroquine concentrates in the highly acidic digestive vacuoles of susceptible plasmodium parasites, where binds to heme and disrupts its sequestration. A parasite-encoded efflux mechanism may account for the reduced levels of chloroquine in the digestive vacuoles of chloroquine-resistant parasites. Residents in malaria endemic areas develop considerably immunity over time, but

pregnancy makes women more vulnerable and infection during pregnancy increases the risk of anemia, miscarriage, stillbirth, and prematurity. The prophylaxis with chloroquine in pregnant women reduces the risk of side effects. The continued use of chloroquine yields a resistance of plasmodium parasites to this drug and the resistance of Plasmodium falciparum to chloroquine is described in numerous reports.

6- CONFLICT OF INTERESTS

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