# THE EFFECT OF MEFLOQUINE CHEMOPROPHYLAXIS ON THE OUTCOME OF PREGNANCY IN WOMEN OF LOW AND HIGH PARITY IN ZARIA, NIGERIA

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## DECLARATION

I hereby declare that the work in the thesis entitled THE EFFECT OF MEFLOQUINE CHEMOPROPHYLAXIS ON THE OUTCOME OF PREGNANCY IN WOMEN OF LOW AND HIGH PARITY IN ZARIA, NIGERIA was performed by me in the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, ZARIA, under the supervision of Prof. I. A. Abdu-Aguye, Prof. A. H. Rafindadi and Prof. I.M. Hussaini. The thesis was not previously presented in whatever form to any institution, organization or anybody other than Ahmadu Bello University, Zaria, Nigeria, for the award of any degree. The information derived from the literature has been duly acknowledged in the text and a list of references provided.

| --- | --- | --- |
| **Name** | **Signature** | **Date** |

## CERTIFICATION

I certify that this thesis entitled: THE EFFECT OF MEFLOQUINE CHEMOPROPHYLAXIS ON THE OUTCOME OF PREGNANCY IN WOMEN OF LOW AND HIGH PARITY IN ZARIA, NIGERIA, carried out by BILKISU BELLO MAIHA, meets the regulation governing the award of Ph.D degree in Pharmacology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## DEDICATION

Dedicated to

My Father, Ambassador Bello Maiha and My Husband, Abubakar Adam

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To my parents, husband, children, brothers and sisters, words cannot express how I feel. Your prayers, encouragement and assistance are what kept me going and helped me see the light at the end of the tunnel. To Fatima Dawoud, only Allah will reward you.

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**ABSTRACT**

Malaria accounts for 25% -70% of infant, 30% of under- five and 7% -11% of maternal mortality in Nigeria. The World Health organization recommended malarial drug prophylaxis for pregnant women in malaria endemic areas to prevent the adverse consequences of malaria in pregnancy. However, the development of resistance by the plasmodium to drugs has led to the failure of such interventions. The current recommended drug regimen for chemoprophylaxis of malaria in pregnancy is two curative doses of sulfadoxine–pyrimethamine (SP), given during the second and third trimesters, but there are reports of resistance to SP in four of the six geopolitical zones of Nigeria. In view of this development, alternative regimens for prevention of malaria in pregnancy have to be investigated to help in policy formulation. This study was designed to study the effect of mefloquine chemoprophylaxis on pregnant women of low and high parity in Zaria, Nigeria. A single blind study involving 985 women in the second trimester of pregnancy randomly distributed, 508 (57.6%) to the mefloquine and 477 (48.4%) to the placebo group, with an average age of 23.77 ± 9.21 was carried out. The mefloquine group received 750mg mefloquine at enrolment and 250mg weekly until delivery while the placebo group received placebo tablets. The effect of mefloquine on haematocrit levels, parasitemia, delivery outcome, anthropometric measurement of babies, placental weight and placental pathology were assessed. Student t- test was used

to compare means and Chi-square test or Fisher's exact test was used to compare proportions. Differences were considered significant at 95%. The placebo group had significantly more women with parasitemia ($p<0.0001$) at enrolment and at weeks 4, and 9 ($p<0.05$, $p<0.005$ respectively), and more reports of fever. None of the women in the mefloquine group developed clinical malaria during the study. The mefloquine group had significantly higher PCV values during the second ($p=0.05$) and third months of the study ($p=0.006$). The high parity mefloquine group also had significantly higher PCV than high parity placebo group at second and third months ($p=0.05$, $p=0.027$ respectively). The mefloquine group had a significantly better delivery outcome than the placebo group ($p=0.027$) with the placebo group having more stillbirths. Incidence and type of congenital malformations (1%) was similar in both groups. High parity mefloquine group had significantly heavier babies ($p<0.05$), larger OFC ($p<0.05$), and heavier female babies ($p=0.022$). The mefloquine group also had heavier ($p=0.011$) females when all parities were combined. Sex had no effect on birth weight within the mefloquine group, while males were heavier than females ($p=0.029$) in the placebo group. Histopathological findings showed that the high parity groups were associated with increased incidence of calcium, fibrin, malaria pigment deposition and malaria parasites in placental tissues. The incidence of nausea was significantly higher in week 4 ($p<0.05$) while vomiting was significantly different in week 1, 2 and 4 ($p<0.05$) for the mefloquine group. There was no significant difference for diarrhoea and pruritus but dizziness was significantly more in the placebo group at week 9 and 16 ($p<0.05$). In conclusion, mefloquine chemoprophylaxis was well tolerated by pregnant women with self limiting adverse effects. They were protected from clinical malaria, had an improvement in haematocrit

levels with an increase in birthweight in the high parity group. Mefloquine was found to be effective for antimalarial prophylaxis in the second half of pregnancy and can be used as an alternative to SP for protection against malaria in pregnancy.

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## ABBREVIATIONS

| | | |
|---|---|---|
| ACPR | - | Adequate Clinical and Parasitological Response |
| ACT | - | Artemisinin Combination Therapy |
| AL | - | Artemether-Lumefantrine |
| BWF | - | Blackwater Fever |
| FP | - | Ferriprotophyrin IX |
| GDP | - | Gross Domestic Product |
| GNP | - | Gross National Product |
| HbF | - | Fetal Haemoglobin |
| H&E | - | Haematoxylin-eosin |
| IE | - | Infected Erythocytes |
| IgG | - | Immunoglobulin G |
| IPT | - | Intermittent Preventive Therapy |
| IUGR | - | Intrauterine Growth Retardation |
| LBW | - | Low Birth Weight |
| MAC | - | Mid-Arm Circumference |
| MPD | - | Mean Parasite Density |
| OFC | - | Occipito-Frontal Circumference |
| PCV | - | Packed Cell Volume |
| PD | - | Parasite Density |
| PFEMP1 | - | *P. falciparum* Erythrocyte Membrane Protein 1 |
| RBC | - | Red Blood Cells |

| | | |
|---|---|---|
| SD | - | Standard Deviation |
| TDR | - | Tropical Disease Research |
| WBC | - | White Blood Cell |
| WHO | - | World Health Organization |
| WRAIR | - | Walter Reed Army Institute of Research |

# CHAPTER ONE

# INTRODUCTION

Malaria is a parasitic disease caused by protozoa of the genus Plasmodium. It is transmitted by the female Anopheles mosquito and is highly prevalent in tropical and subtropical regions of the world, located between latitude 40 degrees north and latitude 30 degrees south of the equator. Due to the high mortality and morbidity associated with it, malaria is considered the most serious of tropical diseases, and a major public health dilemma. Malaria is endemic throughout most of Southeast Asia, the Indian subcontinent, the South Pacific region, Latin America and sub-Saharan Africa.

Three billion, of the world population are at risk, and there are about 350-500 million clinical cases of malaria occurring annually worldwide, with sub-Saharan Africa carrying the greatest burden. About 60percent of the world clinical episodes of malaria occur in sub-Saharan Africa (WHO, 1996 and 2000) and it accounts for about 80% of deaths that occur in the region (Abubakar, 2005).

Malaria is holoendemic in Nigeria and is responsible for enormous economic and health problems. It is one of the four leading causes of mortality in Nigeria (Abubakar, 2005) and contributes to both poverty and under development. Africa looses about 12 billion dollars annually in GDP and Nigeria's GNP is reduced by at least 1per cent annually as a result of malaria (Abubakar, 2005). 132 billion naira is lost annually to malaria, as cost of treatment and loss of man-hours (FMOH, 2005). 50percent of Nigerians experience at

least one episode of malaria/ year. It has been estimated that 71 percent of all expenditure on malaria come from heads of households and 25 percent of household income is expended on malaria. This leads to the vicious cycle of malaria leading to poverty and poverty leading to malaria.

The population groups at risk are pregnant women, infants, and children under the age of five. Malaria contributes to infant and maternal morbidity and mortality. It accounts for 25-70 percent of infant, 30 percent of under- five and 7-11 percent of maternal mortality in Nigeria (Abubakar, 2005; Anekwe, 2005 and FMOH, 2005). Malaria in pregnancy has been shown to account for 2-15 percent of anaemia in pregnancy, 5-14 percent of low birth weight infants and 8-36 percent of preterm deliveries in Nigeria (Anekwe, 2005).

Worldwide, malaria is the most frequent infection affecting pregnant women and poses substantial risk to the mother, her foetus, and the neonate. It has been estimated that each year, 75,000 to 200,000 infant deaths are associated with malaria infections in pregnancy (Steketee *et al*., 2001). It is well recognized that the clinical manifestation of malaria within a community are determined by the degree of endemicity of the infection in the local environment, and by the age-specific levels of immunity acquired through exposure to infection. The clinical effects of malaria in the pregnant woman are also similarly affected. Several studies have shown that the consequences of malarial infections in pregnant women are more frequent and serious in areas of low as opposed to high endemicity. They have shown that acute maternal illness due to heavy parasitemia, leading frequently to maternal death (apparently from cerebral malaria) is more common

in areas of low endemicity (Van-hung, 1951; Menon, 1972) Other consequences of malaria in areas of low endemicity are high rates of abortions, stillbirths, premature deliveries, low birth weight infants, congenital malaria infections and malaria-related anaemia. Moreover, where endemicity is low, women irrespective of parity seem equally affected, but where endemicity is high, the woman who is pregnant for the first time seems most at risk (Brabin, 1983; Nosten *et al*., 1991).

In areas with endemic malaria, most women begin their pregnancies with some level of immunity, and the effects of malaria on pregnancy and its outcome is more difficult to assess (McGregor, 1984). In these areas, malaria increases the risk of maternal anaemia, abortion, stillbirth and low birth weight, particularly in the first pregnancy but the risk diminishes with future pregnancies (Kochar *et al*., 1998). Several studies carried out in hyper endemic areas of Africa, drew attention to the fact that malarial parasitemia is both more prevalent and heavier in pregnant than in non-pregnant women (Bruce-Chwatt, 1952; McGregor and Smith, 1952). McGregor (1984), studied females aged 15-45 years resident in the rural Gambian village of Keneba annually over a 15 year period from 1961-1975. The results showed that malarial parasitemia was significantly more frequent in pregnant than in non-pregnant women ($p<0.01$). Singh *et al*. (1999), also reported similar findings in Central India, where mean parasite densities were significantly higher in pregnant women compared with non-pregnant women for both *P. falciparum* ($p<0.001$) and *P.vivax* ($p<0.05$) infections. In a village in Senegal, pregnancy was associated with a significantly higher prevalence of *P. falciparum* infection, higher

parasite densities, and higher multiplicity of infections. The highest multiplicity of infection was observed in the youngest pregnant women (Scleiermacher *et al.,* 2001).

Pregnancy is also associated with increased susceptibility to malaria. Diagne *et al.* (2000), showed that among women living in areas with high rates of transmission of malaria, the susceptibility to malaria is highest during the second and third trimesters of pregnancy and in the early post-partum period. Studies have shown that primigravidae are more susceptible to malaria than multigravid women with approximately double the infection rate (Archibald, 1956; McGregor, 1984). Bray and Anderson, (1979), and McGregor, (1984), in studies in The Gambia found evidence that susceptibility of pregnant women to malaria changed according to the stage of gestation. The highest prevalence of infection occurring in the second trimester and progressively decline until infection rates at delivery and during the post-partum period, approximate levels in non-pregnant women.

Pregnancy-associated malaria is characterized by placental accumulation of infected erythrocytes. Neonates of mothers with infected placentas have been shown to have lower mean birth weights than neonates of mothers with non-infected placenta (Bruce-Chwatt, 1952; Brabin, 1983). Women who are pregnant for the first and sometimes second time are most likely to have placental infection (McGregor, 1984; Menendez, 2000). The consequence of placental malaria infection is decreased foetal nutrition, as changes in the placenta leads to altered placental integrity, impeding oxygen-nutrient transfer to the foetus. This results in intrauterine growth retardation (IUGR) with

consequent low birth weight, premature delivery, and even foetal death (Gilles *et al.*, 1969; Bruce-Chwatt, 1983).

Malaria also causes severe anaemia in pregnant women especially in primigravidae (McGregor *et al*., 1983; Fleming *et al.*, 1984). The anaemia is as a result of haemolysis of both parasitized and non-parasitized erythrocytes (Juma, 2005) and occurs side by side with other causes of anaemia such as iron and folate deficiency (Fleming *et al*., 1968). This combination of factors worsens the anaemia and indirectly affects foetal growth. Severe maternal anaemia is associated with maternal morbidity, and mortality, premature delivery, low birth weight, and foetal anaemia, which in turn has been shown to be associated with an increased risk of infant mortality (Mahomed, 2000; Menendez, 2000).
The World Health Organization (WHO), recommended malarial drug prophylaxis for pregnant women in malaria endemic areas to prevent the adverse consequences of malaria in pregnancy (WHO, 1984). The drug recommended for use was 5mg/kg body weight chloroquine base weekly (WHO, 1984). Several studies have shown that regular chemoprophylaxis during pregnancy, prevents malaria attack and associated complications like spontaneous abortion, preterm delivery, intrauterine growth retardation (IUGR), and delivery of low birth weight babies (WHO, 1999; Wolfe *et al.*, 2001). However, the development of resistance by the plasmodium parasite to drugs used especially chloroquine, and poor compliance to the chemoprophylactic regimens have led to failure of such interventions over the years.

In Nigeria, pyrimethamine at a dose of 25mg weekly was the most widely used causal prophylactic drug for pregnant women. It is cheap, readily available, and well tolerated. Resistance of *P. falciparum* to pyrimethamine had previously been reported from Northern Nigeria (Archibald 1960; Nahlen *et al.,* 1989) and it was found to be ineffective, and failed to protect pregnant women (Nahlen *et al.,* 1989). Chloroquine 5mg/kg weekly was also used for prophylaxis in Nigeria, but studies have shown that it is ineffective for the treatment and protection of pregnant women against malaria (Okoyeh *et al.,* 1993a; 1993b). Similar findings were reported for other West African countries such as Burkina Faso (Sirima *et al.,* 2003).

Drug efficacy trials conducted in Nigeria in 2002 showed that adequate clinical and parasitological response (ACPR) to chloroquine is less than 75% in all areas of the country except the North West zone which has 77.3% (FMOH, 2005). Sulfadoxine-pyrimethamine also showed less than 75% ACPR in four of the six geo-political zones of the country (FMOH 2005). Countries are advised by the WHO to review their malaria treatment policies if ACPR is less than 75%. This has led to Nigeria reviewing its antimalarial treatment policy, where combination therapy using Artemisinin derivatives and other drugs (ACT) is now the current practice. Artemether-Lumefantrine (AL) is the ACT of choice for treatment of acute malaria, but others such as Artesunate-Mefloquine, Artesunate-Amodiaquine and Dihydroartemisinin-Piperaquine-Trimethoprim can also be used.

The current recommended drug regimen for prevention of malaria in pregnancy is two curative doses of sulphadoxine–pyrimethamine, given during the second and third trimesters with the last dose being given not later than one month before the expected date of delivery (WHO, 2003; WHO, 2004 and FMOH, 2005). This is called the intermittent preventive therapy (IPT). Studies in Kenya and Malawi have shown this intervention to be effective (Shulman *et al*., 1999; Wolfe *et al.*, 2001).

### 1.1 Justification

Most of the anti-malarial drugs that are in use have serious side effects and are toxic to pregnant women and their unborn foetuses. Drugs that are effective against the plasmodium and have few side effects will be useful in the prevention of malaria during pregnancy. No drug for malaria chemoprophylaxis have been tested during pregnancy and consequently travellers and people living in endemic areas can never obtain a full guarantee that any drug is completely free of teratogenic effects. The use of malaria chemoprophylaxis is therefore a balance between risks to the mother from malaria, and a small but unknown risk of adverse reactions in the foetus. The spread of chloroquine and sulphadoxine/pyrimethamine resistance in Africa and Asia means that mefloquine and other drugs such as atovaquone combined with proguanil and doxycycline are the only effective alternatives. Doxycycline is absolutely contraindicated in both the pregnant mother and children below 8 years of age, effectively limiting the choice of pregnant women to mefloquine and atovaquone/proguanil. Mefloquine has been shown to protect pregnant women living on the Thai-Burmese border from malaria without any harmful effect on the foetus when given in the third trimester (Nosten *et al.,* 1990).

This study was designed to study the effect of chemoprophylactic doses of mefloquine on the outcome of pregnancy in our environment. This will help in formulation of policies and in finding alternatives to what is already in use.

### 1.2 Hypothesis

Mefloquine protects pregnant women from malaria

### 1.3 Aim and objectives

**Aim:** To study the effect of mefloquine chemoprophylaxis on the outcome of pregnancy in women of low (first and second pregnancy) and high (third pregnancy and above) parity in Zaria, Nigeria.

**Objectives:**

1. To determine the effect of mefloquine on parasitemia.
2. To determine the effect of mefloquine on outcome of pregnancy by measuring the:
    i. Haematocrit level (packed cell volume per cent), at enrolment and every anti natal clinic (ANC) visit.
    ii. Delivery outcome
    iii. Anthropometry of babies born to women in the study-birth weight, birth length, mid-arm circumference (MAC) and occipito-frontal circumference (OFC).
    iv. Placental weight and histopathology.

3. To assess the safety and tolerability of mefloquine as a chemoprophylactic agent in pregnant women

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Mefloquine

### 2.1.1 History

Mefloquine is a 4-quinoline-methanol derivative related to quinine, discovered in 1971 through a gigantic malaria screening programme of the Walter Reed Army Institute of Research (WRAIR). This programme was necessary because since the 1960s, the malaria situation deteriorated in most tropical areas. This was as a consequence of technical, administrative and financial difficulties encountered by the countries affected, especially in controlling the mosquito vector of malaria. There was also the emergence and spread of strains of *Plasmodium falciparum* resistant to the standard anti-malarial agents. In 1975, the Tropical Disease Research (TDR) scientific working group for the Chemotherapy of Malaria (SWG CHEMAL) decided to co-ordinate and complete the development of the most active schizontocidal compound against chloroquine-resistant strains of *P. falciparum*. Of the many 4-quinoline-methanols that were tested based on their structural similarity to quinine, mefloquine displayed high activity on animal models and emerged from clinical trials as safe and highly effective (Schmidt *et al.*,1978). The drug was then produced by Roche, a Swiss Pharmaceutical Company and registered in 1984 under the trade name Lariam®.

### 2.1.2 Chemistry

Mefloquine is a 4-quinoline-methanol with the specific chemical name of (R*, S*)-(±)-α-2-piperidinyl-2, 8-bis (trifluoromethyl)-4-quinoline methanol. It is a 2-aryl substituted

structural analogue of quinine with the following chemical structure as shown in Figure 2.1. It contains two asymmetric carbon atoms and exists as a racemic mixture of the two erythro enanthiomers (dextro rotatory 11R, 2'S and levo rotatory 11S, 2'R). Both show about the same anti-malarial potency but pharmacokinetic characteristics differ (Martin *et al.*, 1994). When formulated as the hydrochloride, it is a white to almost white crystalline compound, slightly soluble in water with a bitter taste and a molecular weight of 414.78.

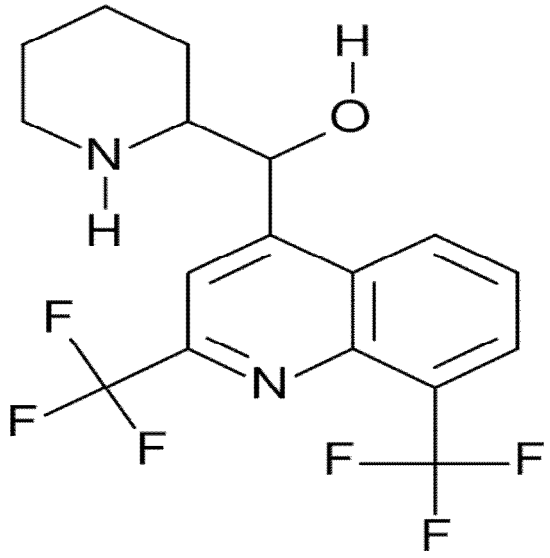



**Fig. 2.1. Chemical structure of mefloquine**

### 2.1.3 Anti-malarial Action

Mefloquine is a blood schizontocide, highly effective against mature trophozoite and schizont forms of all malarial parasites affecting man (*P. falciparum, P. vivax*, *P. ovale*, and *P. malariae*) and also active against the gametocytes of *P. vivax, P. ovale* and *P. malariae*. It has no activity against the hepatic stages of all malarial parasites and mature gametocytes of *P.falciparum* or latent tissue forms of *P. vivax* (Tracy and Webster, 2001)

### 2.1.4 Mechanism of Anti-malarial action

The exact molecular basis of the action of mefloquine is not completely understood. The quinoline-containing anti-malarials are thought to interfere with haemoglobin digestion in the blood stages of the malaria parasite's life cycle. When the asexual malarial parasites digest the haemoglobin in host erythrocytes in their acidic food vacuoles, they release ferriprotophyrin IX (FP) intracellularly. FP is an oxidized form of haeme which is toxic to biological membranes. The parasites are however, not poisoned when they digest haemoglobin because this haeme polymerizes into insoluble unreactive malarial pigment termed haemozoin. Quinoline blood schizontocides that behave as weak bases concentrate in food vacuoles of susceptible plasmodia, where they increase pH, inhibit peroxidative activity of haeme, and disrupt its non-enzymatic polymerization to haemozoin. Failure to inactivate haeme then kills the parasites via oxidative damage to membranes and digestive proteases. Recent kinetic studies indicate that radio labelled chloroquine; quinidine and mefloquine, bind first to haeme and then prevent further polymerization by incorporating as haeme-quinoline complexes into growing haeme polymer chains (Tracy and Webster, 2001).

Malaria-infected red blood cells (RBC) are distinguished from normal cells by their high levels of haeme. The parasite ingests the cytosol of its host cell which is essentially composed of haemoglobin and digests it in its acidic food vacuole (Francis *et al.,* 1997). Whereas parasite proteases hydrolyze globins to its building block (Kolakovich *et al.*, 1997). Free haeme must be detoxified because it is lethal to the parasite (Fitch *et al.*, 1982) and has at least three potential fates in the parasitized RBC:

1) It can be sequestrated into the insoluble haeme polymer hemozoin (HZ) (Slater *et al*., 1991);
2) The haeme that escape polymerization can dissolve into, and translocate across membranes (Rose *et al.,* 1985) reaching the cytosol of the parasite or that of the host cell, where mechanisms exist for its detoxification: or
3) It is possible that some haeme exits the parasitized RBC altogether and binds to serum albumin or to haemopexin (Solen *et al*., 1989).

It is generally assumed that haeme polymerization is the mechanism that protects the parasite from this noxious compound produced during digestion of the host cell haemoglobin. Ginsberg *et al*., (1998) investigated the fate of haeme in *P. falciparum* grown in cultures. They found that the polymerization of haeme is rather insufficient as it amounts to only 20-30%. No free haeme could be detected inside or outside the infected cells which is consistent with the full viability of the parasites, indicating that the parasites are protected from this potentially noxious compound. The mechanism of this protection may be by competitive inhibition of glutathione-dependent degradation of haeme by aminoquinoline anti-malarials such as chloroquine and amodiaquine, thus allowing haeme to accumulate in membranes. Haeme has been shown to disrupt the barrier properties of membranes and to upset ion homeostasis in chloroquine-treated malaria-infected cells.

Mefloquine and Quinine are all lipophilic drugs that bind tightly to serum components, including high-density lipoproteins (Mu *et al.,* 1975; Deaneves *et al*., 1996). This may

facilitate the delivery of mefloquine to the parasites as plasmodia have been shown to accumulate lipids and other hydrophobic molecules from the serum (Berman *et al.*, 1994). Mefloquine also binds with high affinity to membranes (Chevli and Fitch, 1982) and uninfected erythrocytes (Fitch *et al.*, 1979; San George *et al.*, 1984). Photo-affinity labelling studies have identified the erythrocyte integral membrane protein, band 7.2b or stomatin as a mefloquine-binding protein (Desneves *et al.*, 1996). High affinity binding to erythrocytes and other cells may provide a reservoir of mefloquine and contribute to the very long half-life ($T_{1/2}$) of mefloquine in the body (Schwartz *et al.*, 1982).

Mefloquine and Quinine competitively inhibit chloroquine accumulation, and vice versa, probably via a similar mechanism (Fitch *et al.*, 1979; Vander Kooi *et al.*, 1988). Mefloquine and quinine are however much weaker bases than chloroquine and will be monoprotonated under physiological conditions. Uptake of weak bases due to ion trapping is proportional to the square of the charge on the drug and the quinoline methanols are predicted to be concentrated in the food vacuole only by about 200-fold (Yayon *et al.*, 1984, Ginsburg *et al.*, 1989) as compared to chloroquine which is thought to be concentrated several thousand-fold inside the malaria parasite (Aikawa, 1972; Yayon *et al.*, 1984). As a result, mefloquine would not reach the intravacuolar concentration required to inhibit haeme polymerization. Available data suggests that mefloquine interferes with a different step in the parasite-feeding process than chloroquine (Geary *et al.*, 1986). The two high affinity mefloquine-binding proteins in *P. falciparum* infected erythrocytes identified (Desneves *et al.*, 1996), may be involved in mefloquine uptake or action. Despite this, mefloquine is a more potent inhibitor of

growth of drug-sensitive strains of *P. falciparum* than chloroquine (Cowman *et al*., 1994).

Like chloroquine, the quinoline methanols act primarily on the intraerythrocytic asexual stages (Schmidt *et al*., 1978a; Geary *et al*., 1986). Ultra structural studies indicate that mefloquine causes morphological changes in the food vacuole of *P. falciparum*. Mefloquine causes degranulation of hemozoin rather than the clumping of the pigment observed in murine parasites with chloroquine (Jacobs *et al*., 1987; Olliaro *et al*., 1989).

### 2.1.5 Pharmacokinetics

The pharmacokinetics of mefloquine appear to be altered by age (Singhasivanon *et al*., 1994), ethnicity (Palmer *et al*., 1993; Karbwang and White, 1990), pregnancy (NaBangchang *et al*., 1994) and malarial illness (Karbwang *et al*., 1988; Juma and Ogeto, 1989) but these differences do not affect dosing regimens. There is considerable individual variation with respect to plasma or whole blood mefloquine concentrations achieved after the same dose of drug as demonstrated by Karbwang and White, (1990), Pennie *et al*., (1993) and Schwartz *et al*., (2001).

#### 2.1.5.1 *Absorption*

Mefloquine hydrochloride is slowly absorbed from the gastrointestinal (GI) tract when administered as tablets and appears to undergo little, if any first-pass elimination. It is well absorbed in healthy subjects and by patients with uncomplicated malaria following oral administration (Karbwang *et al*., 1987; Looareesuwan *et al*., 1987). In patients with

complicated malaria, adequate blood concentration have been obtained using nasogastric administration, but this route cannot be relied upon for seriously ill patients (Chanthavanich *et al.*, 1985) as absorption may be incomplete (Karbwang and White, 1990). The presence of food significantly enhances the rate and extent of absorption leading to about 40% increase in bioavailability. In a study by Crevoiser *et al.*, (1997) to assess the effect of food on the pharmacokinetics of mefloquine and its major metabolite in healthy volunteers, it was shown that food increases the rate and extent of mefloquine absorption.

The maximum (peak) plasma concentration and area under the curve (AUC) of both mefloquine and its metabolite was higher ($p<0.05$) under post-prandial conditions than under fasting conditions. The time to peak plasma concentration of mefloquine was significantly shorter after food intake (17 hrs Vs 36 hrs) following administration of single oral mefloquine doses of 250-1250 mg in healthy individuals. Peak mefloquine concentrations in the blood or plasma are attained within 6-24 hrs (Median, about 17 hrs) (NaBangchang *et al.*, 1995; Pennie *et al.*, 1993). The maximum concentration (Peak plasma concentration) of mefloquine following the first dose of 250mg was 572 ± 140ug/l (Pennie *et al.*, 1993) and occurred at an average of 17.1 hrs.

The absolute bioavailability of mefloquine has not been determined since parenteral formulation is not available. However, data from a study using radiolabelled drug indicates that the extent of absorption following oral administration of single doses as tablets is about 87-89% of that following administration as an oral suspension

(Looareesuwan *et al.*, 1987). Peak blood or plasma concentrations of mefloquine were observed to be higher in Asians than in other ethnic groups (Palmer *et al.*, 1993).

#### 2.1.5.2 *Distribution*

Mefloquine is widely distributed into body tissues and fluids. It has a large apparent volume of distribution which indicates extensive tissue redistribution. Apparent volumes of distribution of 23.7 ±3.4L in Thai patients with uncomplicated malaria (NaBangchang *et al.*, 1995) to 8.00 ±1.36L in white travellers (Pennie *et al.*, 1993) have been reported. This large apparent volume of distribution appears to be reduced in patients with malaria, including those with uncomplicated disease (Karbwang *et al.*, 1988; Juma and Ogeto, 1989). In children who received mefloquine with sulfadoxine and pyrimethamine as tablets crushed and mixed with glucose syrup, maximum blood-concentrations were higher and were reached at a shorter time compared with equivalent doses in adults (Singhasivanon *et al.*, 1994). Women have higher mean minimum (Cmin ss) and mean maximum (Cmax ss) steady state plasma concentrations than males, apparently due to a generally lower body weight and narrower volume of distribution (Kollaritsch *et al.*, 2000). The apparent volume of distribution however appears to be expanded in pregnancy (NaBangchang *et al.*, 1994), which is consistent with an expanded circulating blood volume and increased tissue binding in pregnancy.

Mefloquine appears to concentrate in erythrocytes to a greater extent than in plasma, binds with high affinity to membranes (Chevli and Fitch, 1982) and uninfected erythrocytes (San George *et al.*, 1984). High affinity binding to erythrocytes and other

cells may provide a reservoir of mefloquine and contribute to its very long half-life in the body (Schwartz *et al.*, 1982). Erythrocyte to plasma concentration ratios of about 2, have been reported in-vitro, while in a study in patients with malaria, the erythrocyte to plasma ratios initially (Parasitemia of 20%) and 3 days later (Parasitemia of 2%) were 4.1 and 1.2 respectively (Baudry *et al.*, 1997).

Mefloquine crosses the blood-brain barrier and has been detected in the CNS. However, because absorption of the drug may be incomplete and erratic in patients with severe malaria, oral mefloquine therapy should not be relied on for CNS infections (Chanthavanich *et al.*, 1985; Karbwang and White, 1990). In a study in rats after administration of 50mg/kg racemic mixture of mefloquine for 22 days, plasma concentration of the (+) enantiomer were more than those of the (-) enantiomer, whereas the opposite was true for every part of the brain. The main metabolite, carboxymefloquine was detected in plasma but not in brain (Baudry *et al.*, 1997).

Small amounts of mefloquine are distributed into breast milk. A single dose study of 250mg Mefloquine in two women indicated that the concentration of mefloquine in milk was only a small proportion of that seen in plasma (Edstein, 1988). Mefloquine also crosses the placenta. According to the manufacturers, it was teratogenic in mice and rats and embryotoxic in rabbits when it was administered at 2-50 times the therapeutic dose in man (Roche, 1994).

#### 2.1.5.3 *Metabolism*

Mefloquine is metabolized by cytochrome P450 isoenzymes of the liver microsomes (Bangchang *et al.,* 1992). Several metabolites are formed but two have been identified in humans. The metabolites appear to be inactive against *P. falciparum* (Hakanson *et al*., 1990). The main metabolite, 2, 8-bis-(trifluoromethyl)-4-quinoline carboxylic acid (carboxymefloquine), has been shown to appear in the plasma 2-4 hours after a single oral dose. The other metabolite, an alcohol, was present only in minute amounts (Schwartz *et al.,* 1982; Bangchang *et al.,* 1992). In a study by Pennie *et al.* (1993), maximum plasma concentrations of carboxymefloquine which were about 50% higher than those of mefloquine, were reached after 2 weeks when 250mg mefloquine was taken weekly. Thereafter, plasma levels of the main metabolite and mefloquine declined at a similar rate. The area under the plasma concentration-time curve (AUC) of the main metabolite was 3-5 times larger than that of the parent drug.

#### 2.1.5.4 *Excretion*

In healthy adults, the mean elimination half life of mefloquine varied between 2 and 4 weeks, with an average of about 3 weeks. Total clearance which is essentially hepatic is in the order of 30ml/min. In volunteers, urinary excretion of unchanged mefloquine and its main metabolite accounted for about 9% and 4% of the dose, respectively. Concentrations of other metabolites could not be measured in the urine (Schwartz *et al.*, 1987). This is consistent with evidence that mefloquine undergoes billiary excretion and extensive enterohepatic circulation in animals. Studies by Karbwang and White (1990) in

animals suggest excretion of mefloquine and its metabolites is primarily in the bile and faeces.

### 2.1.6 Toxicity and side effects

Results of clinical studies and extensive clinical experience indicate that mefloquine when given orally in single doses up to 1500mg or in 250 - 500mg doses each week is generally well tolerated, although certain serious adverse effects have been reported. Females have a higher risk of developing adverse events probably due to a higher dose/ body weight (Phillips and Kass, 1996). The most frequently reported adverse effects are similar to or less severe than those observed with other antimalarial agents (Barrett *et al* 1996; Croft *et al.,* 1997). They include dizziness, nausea, vomiting, diarrhoea, abdominal pain, rash, and pruritus. These side effects tend to be dose related (ter Kuile *et al.,* 1995), self-limiting (Okoyeh *et al.,* 1996; Looareesuwan *et al.,* 1999) and at times difficult to distinguish from the clinical features of malarial illness (ter Kuile *et al.,* 1995; Okoyeh *et al.,* 1996). Long-term prophylaxis with mefloquine has not been associated with an increase in adverse effects (Pennie *et al.,* 1993; Rombo *et al.,* 1993). The frequencies of adverse effects in 134 soldiers, who received mefloquine 250mg weekly for malaria prophylaxis, were diarrhoea 48%, nausea 13%, headache 13%, dizziness 7% and vomiting 2% (Arthur *et al.,* 1990). A series of prospective studies was conducted by ter Kuile *et al.* (1996) to optimize the treatment of multi-drug resistant falciparum malaria on the borders of Thailand between 1990 and 1994, where the tolerance of various treatment regimens containing either 15mg/kg (M15) or 25mg/kg (M25) of mefloquine was evaluated in 3673 patients aged between 6 months and 88 years. Early vomiting (within

1 hour) was an important determinant of treatment outcome despite re-administration of the dose. Overall, 7% of the patients vomited within an hour. Significant risk factors were age ≥6 years or >50 years, higher mefloquine dose (M25), vomiting <24 hours before enrolment, axillary temperature >38.0 C and Parasitemia >10000/ul. In children <2 years, 30% vomited with M25 and 13% did not tolerate a repeat dose. Vomiting was reduced by 40% when the higher dose was split and 50% by giving mefloquine on the second day in combination with artesunate. Anorexia, nausea, vomiting, dizziness, and sleeping disorders were 1.1-1.4 times more frequent with M25 than M15 in the three days following treatment, but were similar in the single or split dose M25 group despite two fold higher mefloquine concentrations obtained with the latter. Dizziness was the most prominent side effect and occurred in 30% of patients treated with the higher dose. 15% complained of vertigo and 5% of the adults were unable to walk unaided. This lasted usually one day and had resolved in all patients by the fourth day.

Diarrhoea also occurred following 15mg/kg or 25mg/kg doses, and following split or single dose. Diarrhoea was related to acute malaria and may thus be unavoidable in a number of mefloquine treated patients. There have been a number of studies which indicated that diarrhoea is an important risk factor of treatment failure following mefloquine administration (Boudreau *et al.,* 1990; Karbwang and white, 1990). There was no evidence however, that diarrhoea, headache, and abdominal pain were associated with mefloquine use (ter Kuile *et al.,* 1996).

Cardiovascular changes, such as bradycardia (<60 heart beat/min) and sinus arrhythmia are commonly reported to the manufacturers and WHO as side effects attributed to mefloquine and have been a consistent finding occurring in up to 68% of the patients in hospital-based studies in Thailand (Harinasuta *et al.,* 1985; Chongsuphajaisiddi, 1987), and Zambia (Ekue *et al.,* 1983). Bradycardia is usually observed 3 to 7 days after the start of treatment when fever has resolved. However profound bradycardia (35-40 heart beats/min) and sinus arrhythmia occur as part of the normal physiological response in healthy subjects at rest and may be particularly frequent in hospital- based studies when previously fit young adults are confined to bed. Comparative treatment studies in Thailand and Zambia reported similar rates of bradycardia in mefloquine treated patients compared with rates in those treated with chloroquine (Ekue *et al.,* 1983), halofantrine (Nosten *et al.,* 1993), or artesunate (Karbwang *et al.,* 1994). Another study comparing the cardio toxicity of mefloquine between patients and healthy volunteers, concluded that the casual relationship with mefloquine is uncertain and postulated that both bradycardia and sinus arrhythmia may be a normal physiological phenomenon to patients recovering from acute malaria (Laothavorn *et al.,* 1992). Mefloquine may or may not induce bradycardia; furthermore, no evidence of heart rhythm disturbances has been associated with mefloquine treatment (Supanaranond *et al.,* 1997; Bindschedler *et al.,* 2000).

Neurological or psychiatric disturbances have been reported with mefloquine use and these include anxiety, depression, sleep disturbances, confusion, hallucinations, acute psychosis, toxic encephalopathy and convulsions. Bem *et al.* (1992) in an overview of spontaneous reports of severe psychotic reactions and convulsions due to mefloquine

prophylaxis from its time of introduction until May 1991, reported that the risk of serious neurotoxicity seem to be higher with the use of mefloquine for treatment than with prophylactic use. Bjorkman and Phillip-Howard (1991) estimated that the risk of this side effect associated with prophylactic use of mefloquine in American, French, and Swedish travellers, is about 1 in 15,000 while Weinke *et al.* (1991) estimated 1 in 13,000 in German travellers. In the German study, which was based on a retrospective analysis, serious neuropsychiatric reactions associated with the use of mefloquine in the treatment of malaria was estimated to occur in 1 in 215 cases i.e., sixty times more frequently than with prophylactic use. In another analysis carried out by Luxemburger *et al.* (1991) where 13,950 supervised treatment doses of mefloquine was given to displaced persons in a malarious area of the Thai-Burmese border over two years, mefloquine was given in the combination form of mefloquine/sulfadoxine/pyrimethamine (MSP or Fansimef®) at a single dose of 15/30/1.5mg/kg and mefloquine (Lariam®) alone at a dose of 25mg/kg (M25). Over the two years, six psychotic episodes (four with MSP and two with M25) and two generalized convulsions (one with each treatment) were attributed to mefloquine use. The overall frequency of serious neuropsychiatric reactions in this study was 57 per 100,000 or 1 per 1,754 treatments. All patients recovered uneventfully. This showed that serious neurotoxicity seems to be nearly ten times more probable after treatment than with prophylactic use of mefloquine (Luxemburger *et al.*, 1991).This might be related to higher blood and brain concentrations of the drug or altered cerebral pharmacodynamics in malaria. The neurological and psychiatric adverse events reported in association with mefloquine prophylaxis were of the same type as those reported with other quinine derivative antimalarials. The precise mechanism of serious neurological and psychiatric

reactions is unknown. The only patient populations identified as having an increased risk of developing these serious reactions to mefloquine are persons with a history of seizures or manic-depressive illness (Bem *et al.*, 1992). Mefloquine prophylaxis should not be prescribed to such patients. Patients with recrudescent infections following initial mefloquine treatment were at a more than 7-fold increased risk of a severe neuropsychiatric reaction when treated again with mefloquine 25mg/kg (ter Kuile, 1994).

A case of hypoglycaemia after mefloquine therapy (1500mg over 2 days) was reported in an AIDS patient with protracted diarrhoea (Assan *et al.*, 1995). Blood glucose levels which were normal before treatment dropped to 2.3mmol/L within a few hours and were corrected by intravenous glucose infusion. Quinine and its isomer quinidine are well known causes of iatrogenic hypoglycaemia due to excessive insulin secretion. The exact mechanism of hypoglycaemia induced by other quinine analogues is unclear. This adverse effect has never been described with mefloquine but it has been shown in animal studies that rat islets of Langerhans exposed to mefloquine invitro, secreted significantly more insulin than control islets (Assan *et al.*, 1995). Hypoglycaemia occurs frequently as a complication of quinine therapy (Padmaja *et al.*, 1999) with an incidence of about 15% and one of the triggering factors is vomiting. The patient in the mefloquine case had diarrhoea; therefore the triggering factor here may be dehydration as a result of diarrhoea.

There has been a report of resurgence of black water fever (BWF) in long-term European expatriates in Africa. Black water fever is a severe clinical syndrome, characterized by intravascular haemolysis, haemoglobinemia, and acute renal failure that is classically

seen in European expatriates chronically exposed to *Plasmodium falciparum* and irregularly taking quinine. BWF virtually disappeared after 1950 when chloroquine superseded quinine in the treatment and prophylaxis of malaria. Twenty-one cases of BWF were seen in France between 1990 through 1999 in European expatriates who lived in sub-Saharan Africa. The triggers of BWF were halofantrine (38%), quinine (24%), mefloquine (24%) and halofantrine or quinine (14%) (Bruneel *et al.,* 2001).

Three cases of ototoxicity characterized by high- frequency sensorineural hearing loss and tinnitus due to mefloquine were reported by Fusetti *et al*. (1999). Only one patient had partial remission of hearing loss after suspension of the drug. None of the patients reported improvement of tinnitus.

## 2.2 Mefloquine use in pregnancy

Safe and effective anti-malarials are required to treat and protect pregnant women from the harmful effects of malaria. Pregnancy increases the risk of malaria causing maternal and foetal deaths, miscarriages and stillbirths (WHO, 2001). No drug for malaria chemoprophylaxis have been tested during pregnancy and consequently travellers and people living in endemic areas can never obtain a full guarantee that any drug is completely free of teratogenic effects. The use of malaria chemoprophylaxis is therefore a balance between risks to the mother from malaria, and a small but unknown risk of adverse reactions in the foetus. The spread of chloroquine resistance in Africa and Asia means that mefloquine and other drugs such as atovaquone combined with proguanil and doxycycline are the only effective alternatives. Doxycycline is absolutely contraindicated

in both pregnant women and children below 8 years of age, effectively limiting the choice of pregnant women to mefloquine and atovaquone/proguanil.

Mefloquine, which has been used mainly for the treatment of multidrug-resistant falciparum malaria, especially in Southeast Asia (WHO, 1983) is now also widely prescribed for prophylactic use in travellers. Teratogenic effects were observed in rats and mice at a dose of 100mg/kg/day, while in rabbits a dose of 80mg/kg/day was teratogenic (Roche, 1990). In mice, rats, dogs, and monkeys, mefloquine was neither mutagenic nor teratogenic at doses that were not overtly toxic (WHO, 1983).

There are no adequate and well controlled studies in pregnant women. However, clinical experience with mefloquine has not revealed an embryo toxic or teratogenic effect at the doses used for treatment or chemoprophylaxis (Roche, 1999). A post-marketing surveillance of prophylactic mefloquine use in pregnancy carried out by Roche, on 1,627 spontaneous reports of women exposed to mefloquine before or during pregnancy, found birth prevalence of congenital malformations to be 4% which was not different from the prevalence observed in the general population. In addition, the congenital malformations observed with mefloquine exposure did not show any specific pattern. The data from this study suggests that the teratogenic effect observed in animals in high doses cannot be applied to humans (Vanhauwere *et al.*, 1998).

A study of the outcome of 72 pregnancies where mefloquine was inadvertently used for prophylaxis by US Army Servicewomen failed to show any teratogenic effects but

reported a high rate of spontaneous abortions (Smoak *et al.,* 1997). Italian women who had taken mefloquine (250mg) as a prophylactic when they were either not pregnant or did not know they were delivered babies with no malformations (Balocco and Bonati, 1990). Mefloquine also seemed safe when used for prophylaxis in pregnant Nigerian women (Fleming, 1990). A double blind placebo controlled study of mefloquine prophylaxis in pregnancy (>20 weeks gestation) was conducted in 339 Karen women living in an area of multidrug- resistant malaria transmission of the Thai-Burmese border by Nosten *et al.,* (1994). Mefloquine prophylaxis was well tolerated although the use of an initial loading dose (100mg/kg) was associated with transient dizziness. There were no significant adverse effects on the mother, the pregnancy, infant survival or development.

A retrospective study of mefloquine treatment during pregnancy in displaced people at the Thai-Burmese border, found a significantly high rate of stillbirths (Nosten *et al.*, 1999). In a small study in Eastern Sudan which involved forty pregnant women in their second and third trimesters, who were given mefloquine 25mg/kg for treatment of falciparum malaria following chloroquine failure, mefloquine was found to be safe. There was no abortion and no congenital abnormality in the newborn children and no maternal death (Adam *et al*., 2004).

From clinical data available, there is no indication that the risk of taking mefloquine in the first trimester of pregnancy is greater than that from any of the other antimalarials (chloroquine, proguanil, and sulfadoxine–pyrimethamine) studied and the risk is considerably lower than that associated with falciparum malaria (Philips-Howard *et al.*,

1998). However, WHO recommends that mefloquine should be avoided during the first four months of pregnancy and pregnancy should be avoided for three months after the use of mefloquine, based on theoretical considerations as there is no concrete evidence that mefloquine is teratogenic (WHO, 2003).

## 2.3 Chloroquine

Chloroquine is a 4-aminoquinoline derivative. It is a blood schizontocidal agent, active against asexual erythrocytic forms of most strains of *P. malariae, P. ovale, P. vivax* and all strains of *P. falciparum.* It is not active against preerythrocytic or exo-erythrocytic forms of plasmodia. It is gametocytocidal for *P. malariae* and *P.vivax,* but has no activity against the gametocytes of *P. falciparum*.

### 2.3.1 Mechanism of Action

The exact mechanism of anti-malarial activity of Chloroquine has not been determined. The 4-aminoquinoline derivatives appear to bind to nucleoproteins and interfere with protein synthesis in susceptible organisms. It intercalates readily into double stranded DNA and inhibits both DNA and RNA polymerase. Chloroquine has been shown to concentrate in the parasites' digestive vacuole, increases the pH of the vacuole, and interferes with the parasites' ability to metabolize and utilize erythrocyte haemoglobin. It has been shown that plasmodia forms that do not have digestive vacuoles and do not utilize erythrocyte haemoglobin, such as exoerythrocytic forms, are not affected by chloroquine.

### 2.3.2 Resistance

Resistance to Chloroquine has been reported with increasing frequency in *P. falciparum.* The incidence of *P. falciparum* malaria resistant to chloroquine varies geographically and has been reported in certain parts of China and Southeast Asia, Central and South America, East west and Central Africa, Oceania. In Nigeria the results of drug efficacy trials carried out in the six geopolitical zones of the country in 2002, indicated that chloroquine gives less than 75% adequate clinical and parasitological response in five of these zones (FMOH, 2005).

The mechanism of plasmodium resistance to 4-aminoquinoline derivatives has not been fully elucidated. In chloroquine-resistant *P. falciparum* malaria, erythrocytes infected with the organism apparently do not concentrate chloroquine and low concentrations of the drug are attained in the digestive vacuole of the parasite. Resistant organisms apparently also develop alternate pathways to utilize erythrocyte haemoglobin.

### 2.3.3 Pharmacokinetics

#### 2.3.3.1 *Absorption*

Chloroquine is rapidly and almost completely absorbed from the gastrointestinal tract following oral administration, and peak plasma concentrations of the drug are generally attained within 1-2 hours. Oral administration of 310mg of chloroquine daily results in peak plasma concentration of 0.125µg/ml. Absorption of chloroquine is unaffected by the presence of food in the gastrointestinal tract and may even increase its bioavailability.

#### 2.3.3.2 *Distribution*

Chloroquine is widely distributed into body tissues. The drug has an apparent volume of distribution of 116-285L/kg in healthy adults. Animal studies indicate that concentrations of chloroquine in liver, spleen, kidney and lungs, are at least 200-700 times higher, while concentrations in the brain and spinal cord are at least 10-30 times higher than those in plasma. Chloroquine binds to melanin-containing cells in the eye and skin; concentrations being several times higher than plasma concentrations. The drug is also concentrated in erythrocytes and binds to platelets and granulocytes. Serum concentrations are usually higher than plasma concentrations, which may be as a result of the drug being released from platelets during coagulation. Chloroquine readily crosses the placenta and following oral administration of a single 300 or 600mg dose, peak concentrations of the drug in milk range from 1.7-7.5µg/ml and is generally greater than concurrent plasma concentration. Chloroquine is 50-65% bound to plasma proteins.

#### 2.3.3.3 *Elimination*

The plasma half-life of Chloroquine in healthy individuals is 72-120 hours. Chloroquine is partially metabolized, and the main metabolite is desethyl chloroquine (Gustafson *et al*., 1983). Desethylchloroquine has slightly less antiplasmodial activity, than chloroquine. Bisdesethylchloroquine, a carboxylic acid derivative and several other unidentified metabolites are also formed in small amounts. Chloroquine and its metabolites are slowly excreted by the kidney, by both glomerular filtration and tubular secretion (Gustafsson et *al.,* 1983). Up to 70% of a dose is reportedly excreted unchanged

while up to 25% of the dose may be excreted as desethylchloroquine in urine (Walker *et al.,* 1986; Gustafsson *et al.,* 1983).

### 2.3.4 Uses

Chloroquine is used for the treatment of malaria caused by sensitive strains of *P. falciparum* and treatment or prophylaxis of malaria caused by *P. malariae, P. ovale, and P. vivax.*

## 2.4 Atovaquone

Atovaquone is a hydroxynaphthoquinone derivative. It is a blood schizontocide active against erythrocytic stages of most strains of plasmodia specie. It is active against exoerythrocytic forms of plasmodium and early gametocyte but lack activity against *P.vivax* hypnozoites. Atovaquone is also active against a variety of protozoa including *Pneumocystis carinii,* and *Toxoplasma gondii* (Hughes *et al.,*1990; Hudson *et al.,* 1991).

### 2.4.1 Mechanism of Action

The exact mechanism of antiprotozoal action has not been fully elucidated. Atovaquone interferes with the biosynthesis of pyrimidines of malarial parasites. It selectively inhibits mitochondrial electron transport, reduces pyrimidine biosynthesis and collapses mitochondrial membrane potential, thereby preventing parasite replication.

### 2.4.2 Adverse effects

Although adverse effects associated with atovaquone therapy are common, the drug generally appears to be well tolerated. The most frequent adverse effects reported in 5% or more of patients include abdominal pain, nausea, vomiting, diarrhoea, fever, headache, asthenia, anorexia, dizziness, pruritus and rashes.

### 2.4.3 Uses

Atovaquone is used with proguanil in adults and children weighing 11kg or more for suppression or chemoprophylaxis of malaria, caused by *P. falciparum* including chloroquine-resistant strains of *P. falciparum*. It is also used for treatment of acute uncomplicated malaria infection by *P. falciparum*. This combination is considered as one of the drugs of choice for chemoprophylaxis for travellers to areas where chloroquine-resistant *P. falciparum* malaria is endemic along with mefloquine and doxycycline. Results of a clinical study showed response rates of 94-100% in adults and children 2years of age or older with falciparum malaria (Looareesuwan *et al*., 1999).

## 2.5 Artemisinin and its Derivatives

Artemisinin is a sesquiterpene lactone endoperoxide derived from the weed qing hao (*Artemisia annua*), also called sweet wormwood or annual wormwood. The Chinese have described medicinal value of this plant for more than 2000 years. In 1972, Chinese scientists extracted and crystallized the major antimalarial ingredient, qinghaosu, now known as Artemisinin. Three derivatives with greater antimalarial potency than

artemisinin, were synthesized, namely dihydroartemisinin, artemether and artesunate (Tracey and Leslie, 2001).

### 2.5.1 Anti malarial Action

Artemisinin and its derivatives are potent and rapidly acting blood schizontocides active against all *Plasmodium* species. They act rapidly upon asexual erythrocytic stages of *P. vivax* and chloroquine-sensitive, chloroquine-resistant and multidrug-resistant strains of *P. falciparum.* Their potency in-vivo is 10 to 100 times greater than that of other antimalarial drugs (White, 1997). They have gametocytocidal activity but do not affect either primary or latent tissue stage parasites. Thus artemisinin derivatives are not useful either for chemoprophylaxis or for preventing relapse of vivax malaria. They reduce the gametocyte carriage and thus transmission of malaria which contributes to the control of malaria in areas of low endemicity.

### 2.5.2 Mechanism of Action

The endoperoxide moiety is required for antimalarial activity of artemisinin and its derivatives. The mechanism of action involves two steps. First, intraparasitic haeme iron of infected erythrocytes catalyzes cleavage of the endoperoxide bridge in the artemisinins. This is followed by intramolecular rearrangement to produce carbon-centered radicals that covalently modify and damage specific malarial proteins (Meshmick *et al.*, 1996).

### 2.5.3 Pharmacokinetics

Time to peak plasma levels for the artemisinin derivatives varies from minutes to several hours, depending on the drug formulation and its route of administration. Likewise, the profile and extent of drug binding to plasma proteins, is variable. Artemether and artesunate are both converted to dihydroartemisinin, which is an active metabolite (de Vries and Dien, 1996). Artemisinin itself is metabolized to at least four inactive metabolites, although it is unclear whether dihydroartemisinin is formed as an intermediate. The antimalarial effect of artemisinin compounds results primarily from dihydroartemisinin, which rapidly disappears from the plasma with a half life of about 45 minutes. Little or none of the administered drugs or dihydroartemisinin is recovered in urine. Artemisinin induces CYP2C19 in humans (Svensson *et al.*, 1998), however, there is no evidence yet of clinically important drug interactions as a consequence.

### 2.5.4 Toxicity and Contraindications

Artemisinin derivatives given for up to seven days at therapeutic doses appear to be safe in humans (de Vries and Dien, 1996). Transient first-degree block, dose related reversible decreases in reticulocyte and neutrophil counts, and temporary elevations of serum aspartate aminotransferase activity have been reported, but clinical significance is not established. Brief episodes of drug-induced fever in human volunteers were noted in some studies but not in others.

High doses of artemisinin derivatives can produce neurotoxicity, prolongation of the QT interval, bone marrow depression and foetal resorption in experimental animals,

consequently the possibility of long-term toxicity in human beings exists (de Vries and Dien, 1996). However evidence thus far indicates that artemisinin derivatives are remarkably safe for emergency treatment of severe, multidrug resistant malaria.

### 2.5.5 Therapeutic Uses

Artemisinin derivatives are the most rapidly acting, effective, and safe drugs for the treatment of severe malaria, including infection due to chloroquine and multidrug-resistant strains of *P. falciparum* (White and Olliaro, 1998). They are effective against cerebral malaria but should not be used for prophylaxis. Although they can be used as single agents, infections often relapse unless therapy is continued for 5 to 7 days. To delay the development of resistance and prevent relapse, they should be given with longer acting drugs such as the quinolines (e.g. mefloquine) or antibiotic (doxycycline) antimalarials (White, 1999).

### 2.5.6 Artemisinin Based Combination Therapy

To counteract the threat of resistance of *P. falciparum* to monotherapies, and to improve treatment outcome, combinations of antimalarials are now recommended by World Health Organization (WHO) for the treatment of falciparum malaria. The drug combined should be blood schizontocides with independent modes of action and thus unrelated biochemical targets in the parasite. The rationale for combining antimalarials with different modes of action is two fold:

1. The combination is often more effective and

2. In the rare event that a mutant parasite that is resistant to one of the drugs arises, during the course of the infection, the parasite will be killed by the other drug. This mutual protection is thought to prevent or delay the emergence of resistance.

The currently recommended ACTs are: Artemether-Lumefantrine, Artesunate-Amodiaquine, Artesunate-Mefloquine, and Artesunate-Sulphadoxine-Pyrimethamine.

Three day course of these combinations are effective and superior to monotherapies. In these three day ACT regimens, the artemisinin component reduces the number of parasites in the body by a factor of approximately one hundred million, and complete clearance of the parasite is dependent on the partner medicine being effective and persisting at parasiticidal concentration until all the parasites have been killed. Consequently, the artemisinin component is protected from resistance by the partner medicine provided it is efficacious and the partner medicine is partly protected by the artemisinin derivative.

**2.6 Anaemia in Pregnancy**

The World Health Organization (WHO) defines anaemia in pregnancy, as a haematocrit value of below 36% (<11.0g/dl). A study by Lawson (1967) showed that significant harm to the foetus and mother does not occur until haemoglobin value was below 10g/dl or a haematocrit value less than 30%. As a result of this, many hospitals in Nigeria use haemoglobin level of 10g/dl or less as indicating anaemia. In malaria infestation, both parasitized and non-parasitized red cells are haemolysed.

Anaemia in pregnancy is defined as a haematocrit value below 30% (Sowunmi, 2003). Anaemia in pregnancy is common in Nigeria and its incidence ranges from 30-60% (Sowunmi, 2003). There are several causes of anaemia in pregnancy, but the commonest include iron and folic acid deficiency (Ogunbode and Oluboyede, 1986) and malaria infection (Fleming *et al.,* 1984).

**2.7 Placental Malaria**

This is a complication of malaria and its incidence in Nigeria has been reported to be 23-40% (Bruce-Chwatt, 1952; Sowunmi, *et al.,* 1996). Placental parasitemia is more frequent and heavier than maternal peripheral parasitemia, with about 50% of women having placental parasitemia not showing peripheral parasitemia (McDermott *et al.,* 1988). The foetus is protected from malarial infections by passive immunity acquired from trans-placental transfer of maternal immunoglobin G (IgG) antibodies, and the presence of the placental barrier which physically limits the passage of parasites into the foetal circulation. It is also well documented that foetal haemoglobin (HbF), hinders the development of the malaria parasite. A hypothetical model in which recirculation of memory T-lymphocytes from the intervillous blood to local lymphoid tissue facilitates maintenance of local memory immunity in malaria is described by (Moore, *et al.,* 2000). This hypothesis explains how memory cells might be retained when the placenta is expelled at parturition and thus remains available for rapid recall from the local lymphoid tissue to the intervillous space when exposure to the same antivillous stimulus occurs in subsequent pregnancies.

Infections with *P. falciparum* during pregnancy lead to the accumulation of parasitized red blood cells (Infected erythrocytes, IEs) in the placenta. IEs of *P. falciparum* isolates that infect the human placenta were found to bind IgG. A strain of *P. falciparum* cloned for IgG binding, adhered massively to placental syntiotrophoblasts in a pattern similar to that of natural infections. Adherence was inhibited by IgG-binding proteins, but not by glycosaminoglycans or enzymatic digestion of chondroitin sulphate or hyaluronic acid. Normal non-immune IgG that is bound to a duffy binding-like domain of the *P. falciparum* erythrocyte membrane protein 1 (PFEMP1) might at the IE surface act as a bridge to neonatal FC receptors of the placenta. The accumulation of IEs carrying IgG in the placenta and their absence from the peripheral circulation suggests that *P. falciparum.* IEs with IgG-binding phenotype are selected for in the placenta.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Study population

A survey of women of reproductive age was carried out in some wards of Zaria city. Based on this survey, seven wards were chosen for the study. The criteria used for the choice of wards were:

1. Proximity of the ward to the health centre used for enrolment and
2. Availability of a community health worker who resides or works in the ward to follow up the pregnant women.

The wards are; Limanchi, Banzazzau, Fatika, Nufawa, Juma, Magajiya, and Kaura.The location of these wards are shown in Appendix I.

Two centres, General Hospital, Kofan Gayan (now Hajiya Gambo Sawaba General Hospital) and the Babban Dodo Health Centre, both in Zaria city were chosen for the study. Pregnant women from these wards were enrolled into the study based on the following criteria:

1. Pregnancy of 12 to 24 weeks gestation.
2. No history of intake of anti malarial drugs for the previous four weeks.
3. Resident in the chosen wards.
4. Written consent from their husbands to participate in the study ( consent form in Appendix II).

#### 3.1.1.1 *Study group*

Pregnant women who satisfied the criteria of eligibility and were attending the antenatal clinic for the first time were enrolled into two groups: the study group and the control group. Enrolment into the two groups were done on a weekly rotatory basis, for example, if enrolment in week 1 of October was mefloquine, week 2 would be placebo, week 3 mefloquine, week 4 placebo etc, and even if enrolment was not done for a particular week, the arrangement is maintained. The women in each group were further subdivided into high parity and low parity. High parity, were women carrying their third pregnancy and above, while low parity were women who were pregnant for the first or second time.

- ***Mefloquine Group***

The women in this group received 750mg (3 tablets) of mefloquine on the day of enrolment followed by 250mg (one tablet) of mefloquine weekly until delivery under supervision.

- ***Control group***

These women received 3 tablets of a placebo on the day of enrolment and one tablet weekly until delivery under supervision. The placebo tablet looked exactly like the mefloquine tablet and the women could not differentiate between the two if they were placed side by side.

All women in the two groups received monthly supplies of 200mg ferrous sulphate and 5mg folic acid for daily intake.

### 3.1.2 Criteria for withdrawal from Study

Any woman who had any of these reasons was withdrawn from the study.

1. Hypersensitivity to mefloquine.
2. Non-compliance for 3 weeks or more.
3. Unwillingness of either the woman or her husband to continue with the study.
4. High risk pregnancies.
5. Lack of good and complete follow-up.
6. Moving out of designated wards or travelling out of town for more than two weeks.

### 3.1.3 Protection of human subjects

Approval for conducting the study in Nigeria was sort and was given by the Nigeria Combating Childhood Communicable Diseases, Research Review Committee, at its ninth meeting in Lagos, Nigeria (Appendix III). The approval for the use of mefloquine as the chemoprophylactic agent was given by the WHO (Appendix IV).

### 3.1.4 Chemicals and reagents

- Potassium dihydrogen ortho phosphate ($KH_2$ $PO_4$), EDH Chemical Ltd, Poole, England. Batch Number 1884380.
- Disodium hydrogen orthophosphate ($Na_2$ $HPO_4$), EDH Chemical Ltd, Poole, England. Batch Number 6539490.
- Giemsa stock solution was obtained from the National Malaria and Vector Control Division, Federal Ministry of Health, Abuja, Nigeria.

- Ethanol (Absolute), May and Baker, Ltd., Degenham, England.
- Xylene (Sulfur-free), May and Baker, Ltd., Degenham, England.
- Methanol, R-grade (Re-sublimed) May and Baker, Ltd., Degenham, England.

### 3.1.5 Equipments

- Digital Baby Scale, Model 727, Made in England.
- Rollametre, Raven Equipments LMT, Made in England.
- Vacutainer Tubes, England.
- Glassware.

## 3.2 Methods

### 3.2.1 Study methods

#### *3.2.1.1 Information obtained on Enrolment*

Information about the personal, past obstetric and medical history of the enrolees were collected and entered on a form as shown in Appendix V.

#### 3.2.1.2 *Parasitological outcome*

Women in the placebo group who had parasitemia on enrolment were given a full course of chloroquine (1500mg), while those in the mefloquine group were not given any as they received 750mg of mefloquine which cleared their parasitemia. Any woman who developed parasitemia during the follow-up period was given a full course of chloroquine, for treatment.

### 3.2.2 Preparation of blood films for Malaria parasite count

- ***Thin Film***

The finger prick method was used. The finger tip was swabbed clean with disposable alcohol swab and left to dry. A sterile autolet was used to prick the finger which was then gently squeezed. The first drop was cleaned off with sterile dry cotton wool and a second drop transferred to the middle of a well cleaned microscope slide. A second slide was held at an angle of $45^{o}$ and the blood was spread along the length of the slide to give a thin film.

- ***Thick Film***

Another drop of blood was put at the end of the same slide and an oblong shape was made using the edge of a second slide. The slide was then labelled with the ID number and date or week of enrolment, and the film was left to dry.

- ***Staining of blood film***

Blood films were stained for 30mins with 4% Giemsa solution, after which a buffer of pH 6.2 was added to the staining fluid on the slide to the point where overflowing was just prevented. This was allowed to stay for 30sec after which the slides were then drained, rinsed with buffer solution (pH 6.2) and air dried.

### 3.2.3 Parasite Counting

Stained slides were examined, using a standard x 100 oil immersion objective and x 10 eye piece, to give a total magnification of x 1000. For screening and definitive counting of thick films the ″farmer ploughing″ method was used, while the ″battlement″ technique was applied to examine thin films (WHO, 1984). A slide was considered negative only after about 100 microscope fields had been examined.

To determine parasite density, a field was focused and the malaria parasites and leucocytes in that field were counted and recorded with two handed tally counters. One counter was used for counting of malaria parasites and the other for white blood cells. The counting process ended when the parasite count reached 500 or the leucocytes 1000, depending on which of the figures was reached first. In the process of counting, these figures may be attained before counting of a particular field was completed. In such a case counting was continued until all the parasites and leucocytes in that particular field had been counted.

- ***Determination of parasite density (PD) per $mm^3$ of blood***

The parasite density was determined by dividing the product of the parasite count and 6,000, by the leukocyte count obtained. The number 6,000 standing for the standard white blood cell count in this environment. The formula (WHO, 1988) is given below:

$$PD\backslash mm^3 = \frac{\text{Number of parasites counted} \times \text{Standard leukocyte count /mm}^3 \text{ of blood}}{\text{Number of leucocytes counted}}$$

- ***Determination of Mean Parasite Density (MPD)***

The mean parasite density of women who were positive for each week was determined by dividing the sum of their parasite densities by the total number of women with positive parasitemia.

$$\text{MPD week 1} = \frac{\sum \text{PD/mm}^3 \text{ of week 1}}{\text{Number of women with positive Parasitemia in week 1}}$$

### 3.2.4 Total White Blood Cell (WBC) Count

The Bulk Dilution method was used. 0.4ml of filtered Twerks solution (WBC diluting fluid) was put in a test tube and 0.2ml of well mixed blood was added to it using 0.2ml micropipette. The suspension was well mixed by taping the bottom of the tube. The counting chamber and cover glass were cleaned and placed on a flat surface. Using firm pressure, the cover glass was slid over the counting chamber until a rainbow effect on both sides of the cover slip was seen (Neuton ring). The chamber was then filled using a Pasteur pipette filled with the well mixed blood suspension by touching the edge of the cover glass. The chamber was then placed on a microscope stage and the cells allowed settling for two minutes. Using a 4mm objective, and x10 eye piece, the objective was focused on to the four corners of the square. The cells in the four corners were counted. The total WBC count was then calculated.

### 3.2.5 Differential White Blood Cell Count

A drop of heparinized blood was placed on a microscope slide and spread out along the length of the slide held at an angle of $45^{o}$. The film was air dried and stained with

Leishman stain. The stain was put on the film, left for 2 minutes and then buffered distilled water (pH 6.8) was added to the staining fluid on the slide until overflowing was just prevented. The slide was drained after 8 minutes, rinsed with tap water and was then air dried. Using a multiple counter, the number of polymorphornuclear and mononuclear leucocytes were counted up to 100, using an oil immersion lens, under a microscope with total magnification of 800 times. The cells counted were neutrophils, eosinophils, basophils, lymphocytes and monocytes. Each count was recorded as a percentage.

$$= \frac{\text{Number of individual cells}}{\text{Total number of WBC}} \times 100$$

### 3.2.6 Packed Cell Volume (PCV)

A heparinized PCV tube was filled to 3/4 of its length with blood, and the unfilled end sealed using a burnsen burner. The sealed tubes were then put in a micro-haematocrit centrifuge and spun at 3000rpm for 5 minutes. The height of the red blood cells was read using a micro-haematocrit reader on a flat surface and the reading, expressed as a percentage.

### 3.2.7 Histopathology of the placenta

After delivery, thick and thin blood films were made with blood from the maternal surface of the placenta and cord blood and these were labelled. 5cm of the cord was measured and the rest was cut off. The placenta was then examined to see if it was complete or not. The placenta was rinsed in water to make it clear for observation and to prevent the reaction of blood and formalin which produce a pigment similar to malaria pigment. After washing, the placenta was then weighed and examined. Blocks were

taken from any pale grey, white areas on the maternal surface. About 2-3cm of the placenta tissue was taken and then cut into 15 blocks of 2-3cm, for easy fixing. The blocks were then washed again and stored in labelled bottles filled with freshly buffered solution formalin. The placenta tissues were cut using a microtome into 5µm thick sections and slides were prepared using these sections. The slides were stained with haematoxylin-eosin (H&E) and viewed under a microscope.

### 3.2.8 Sample size and data analysis

A sample of about 500 pregnant women per group was needed making a total of 1000 women. Data were analyzed using Statistical Package for Social Science (SPSS) 11.0. The student t-test was used for analysis of quantitative data and Chi Square or fisher's exact tests were used for proportions. Differences were considered significant at $p < 0.05$

# CHAPTER FOUR

# RESULTS

## 4.1 General characteristics

A total of 1027 women were enrolled into the study but 42 of them dropped out after the day of enrolment leaving 985. They were randomly distributed to two groups, 508(57.6%) to the mefloquine group and 477(48.4%) to the placebo group. They were further subdivided into low and high parity groups with the high parity groups making up 60% of the study population (Table 4.1). There was no statistical difference in age between the two groups with an average age of 23.15±6.22, range (12-51yrs), (Table 4.2). The study population was mainly made up of Hausa/ Fulani women (95.6%). This agreed with the location of the study in Zaria City which is a predominantly Hausa/ Fulani community, and the distribution of other ethnic groups in the study groups were similar.

The height, body weight and mid arm circumference, were similar in both groups at the time of enrolment while the triceps skin fold was significantly (p=0.046) thicker for the high parity mefloquine group (Table 4.2).

**Table 4.1: Distribution of pregnant women in the study by study group and parity**

| | Parity | | |
|---|---|---|---|
| **Study Group** | **Low Parity** | **High Parity** | **Total** |
| **Mefloquine** | 210(41) | 298(59) | 508(51.6) |
| **Placebo** | 186(39) | 291(61) | 477(48.4) |
| **Total** | 396(40) | 589(60) | 985(100) |

**$X^2$ = 0.563, df= 1, p= 0.453**

- **Numbers in parenthesis are percentages**

**Table 4.2: General characteristics of pregnant women at time of enrolment into the study**

| Characteristic | Study group | Parity | | Total |
|---|---|---|---|---|
| | | Low parity | High parity | |
| **Age (years)** | **Placebo** | 18.41±2.52(184) | 26.75±6.18(288) | 23.50±6.51(472) |
| | **Mefloquine** | 18.27±2.67(208) | 26.83±5.49(297) | 22.83±5.93(505) |
| **Body Weight (kg)** | **Placebo** | 49.39±6.83(179) | 52.83±9.56(277) | 51.45±8.74(455) |
| | **Mefloquine** | 49.78±7.89(203) | 53.67±8.98(286) | 52.08±8.61(490) |
| **Height (cm)** | **Placebo** | 156.06±6.13(167) | 155.50±6.34(256) | 155.72±6.26(423) |
| | **Mefloquine** | 156.04±6.22(187) | 156.64±5.41(254) | 156.39±5.76(437) |
| **Mid-arm** | **Placebo** | 24.40±2.68(170) | 25.66±4.01(259) | 25.16±3.59(429) |
| **circumference(cm)** | **Mefloquine** | 24.09±3.88(205) | 25.86±3.94(278) | 25.11±4.00(483) |
| **Triceps skin fold** | **Placebo** | 13.63±5.77(154) | 14.85±6.89(226) | 14.35±6.48(380) |
| **(mm)** | **Mefloquine** | 14.02±5.39(193) | 16.12±7.00(257)***** | 15.22±6.44(450) |

- **Number in parenthesis are sample size** *** p=0.046**
- **Data are presented as Mean ± SD**

### 4.1.1 History of Previous Pregnancies

The mean number of previous pregnancies was 2.94±2.87, with a maximum of 14 pregnancies (Table 4.3). The women reported a history of 183 (21.3%) abortions, 71(7.2%) stillbirths, and 93 (9.4%) neonatal deaths. About 24.5% of all the women enrolled were primigravidae while, 15.7% were secundigravidae. This gave a total of 40.2% of the study population being of low parity (Appendix VI).

**Table 4.3: Distribution of study groups by history of previous pregnancies**

| Study Group | | Previous Pregnancies | No. of live Births | No. of Abortions | No. of Still Births | No. of Neonatal Deaths |
|---|---|---|---|---|---|---|
| **Mefloquine** | Mean±SD | 2.77±2.74 | 2.34±2.42 | 0.21±0.55 | 0.07±0.27 | 0.11±0.40 |
| | N | 506 | 496 | 496 | 496 | 496 |
| | Minimum | 0 | 0 | 0 | 0 | 0 |
| | Maximum | 13 | 11 | 4 | 2 | 3 |
| **Placebo** | Mean±SD | 3.12±3.00 | 2.50±2.56 | 0.33±0.74 | 0.10±0.33 | 0.14±0.44 |
| | N | 474 | 462 | 462 | 462 | 462 |
| | Minimum | 0 | 0 | 0 | 0 | 0 |
| | Maximum | 14 | 11 | 6 | 3 | 3 |
| **Total** | Mean±SD | 2.94±2.87 | 2.42±2.49 | 0.27±0.65 | 0.08±0.30 | 0.13±0.42 |
| | N | 980 | 958 | 958 | 958 | 958 |
| | Minimum | 0 | 0 | 0 | 0 | 0 |
| | Maximum | 14 | 11 | 6 | 3 | 3 |

**N= sample size**

### 4.1.2 Haemoglobin Genotype

The genotype of all the pregnant women enrolled in the study was determined except for 193 (19.6%) of them. 77.4% of the women were AA, 22.5% were AS and only one (0.1%) was SS. The woman with sickle cell disorder (SS), belonged to the placebo group. Table 4.4, shows the distribution of the genotypes of the pregnant women by study group.

**Table 4.4: Haemoglobin genotype of pregnant women taking mefloquine prophylaxis**

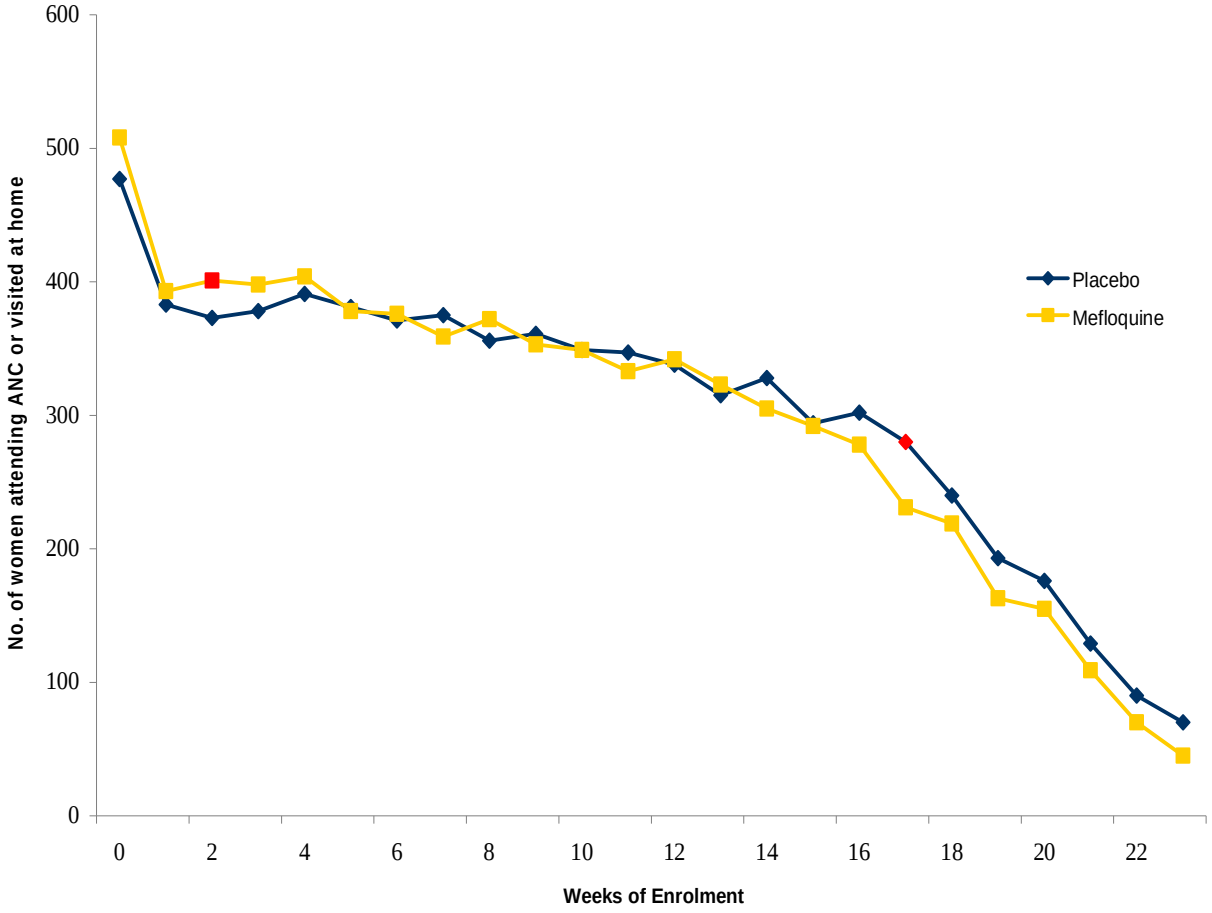
| | HB Electrophoresis | | | |
|---|---|---|---|---|
| **Study Group** | **AA** | **AS** | **SS** | **Total** |
| **Mefloquine** | 298(73.2) | 109(26.3) | | 407(100) |
| **Placebo** | 315(81.8) | 69(17.9) | 1(0.3) | 385(100) |
| **Total** | 613(77.4) | 178(22.5) | 1(0.1) | 792(100) |

- **Numbers in parenthesis are percentages**

### 4.1.3 Attendance of ANC and weekly home visits

All pregnant women enrolled in the study were seen once a week, when drugs were administered, blood samples for making films taken, and questions asked about their general wellbeing. Those that came for ANC were seen in the clinic, while those not due for ANC were visited at home. Many women were lost to follow up because they stopped coming for ANC, or they were not always at home when visited by the community health officer.

The number of women seen every week in each group was compared, and there was no significant difference between the placebo and mefloquine groups for the 23 weeks analyzed except in week 2, where the mefloquine group had significantly ($p<0.05$) more women being attended to for the week, and week 17, where the placebo group had significantly higher attendance ($p<0.05$). This showed that the number of women seen each week were comparable for both groups except for those two weeks. The attendance progressively decreased as the weeks increased because of deliveries as shown in Fig 4.1 and Appendix VII.




- **Red colour indicates significance at p= 0.05**

**Fig. 4.1: Number of women in the study who attended ANC or was visited at home**

## 4.2 Social Characteristics

### 4.2.1 Alcohol Consumption

Consumption of alcohol during pregnancy can cause foetal alcohol syndrome, which is characterized by low birth weight, mental retardation, and congenital heart disease. Only two (0.2%) women belonging to the mefloquine group admitted drinking alcohol as shown in Table 4.5.

**Table 4.5: Alcohol consumption by pregnant women in the study groups**

| | Alcohol Consumption | | |
|---|---|---|---|
| **Study Group** | **Yes** | **No** | **Total** |
| **Mefloquine** | 2(0.4) | 461(99.6) | 463(100) |
| **Placebo** | 0 | 448(100.0) | 448(100) |
| **Total** | 2(0.2) | 909(99.8) | 911(100) |

- **Numbers in parenthesis are percentages**

### 4.2.2 Smoking

Smoking during pregnancy has been associated with spontaneous abortion, premature delivery, and low birth weight, with babies born having slower initial growth. The pregnant women enrolled in the study were asked about their smoking habits, three (3) women (0.6%) of the mefloquine group are smokers, while there was none in the placebo group (Table 4.6).

**Table 4.6: Smoking of Cigarettes by pregnant women in the study groups**

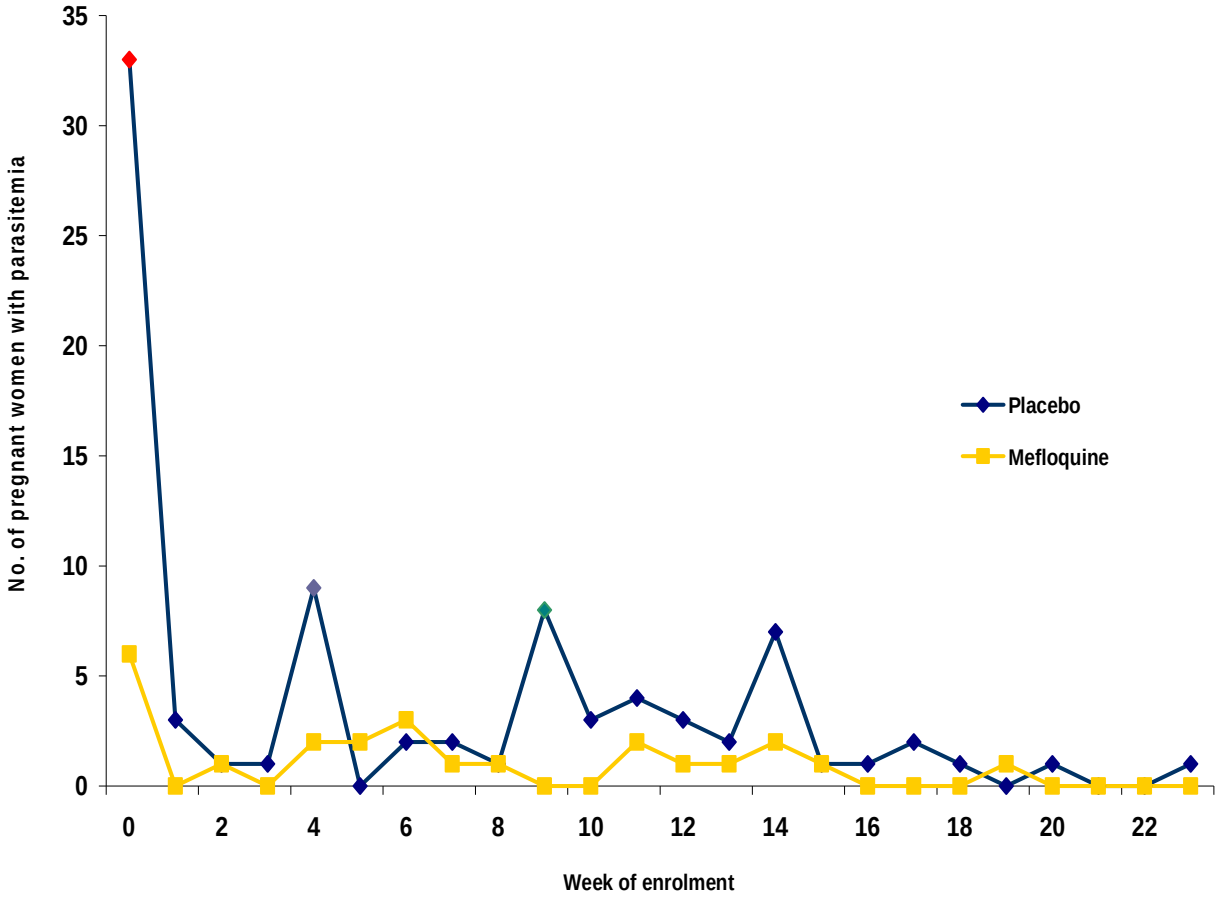
| | Cigarette Smoking | | |
|---|---|---|---|
| **Study Group** | **Yes** | **No** | **Total** |
| **Mefloquine** | 3(0.6) | 460(99.4) | 463(100) |
| **Placebo** | 0 | 448(100) | 448(100) |
| **Total** | 3(0.3) | 908(99.7) | 911(100) |

- **Numbers in parenthesis are percentages**

### 4.3 Effect of mefloquine on Malaria infection

#### 4.3.1 Effect of mefloquine on parasitemia

Blood films for malaria parasite counts were made every week for all pregnant women in the study. At enrolment, it was observed that, the placebo group had significantly more women with parasitemia ($p=0.0001$) than the mefloquine group. About 7% of the women enrolled in that group had detectable malaria parasites in their blood as compared to 1% of the mefloquine group. Those found to be parasitemic in the placebo group, were treated with a full course of chloroquine 25mg/kg/body weight over three days. The placebo group also had significantly more women with parasitemia, at weeks 4, and 9 ($p=0.03$, $p=0.008$ respectively) (Fig.4.2 and Appendix VIII).

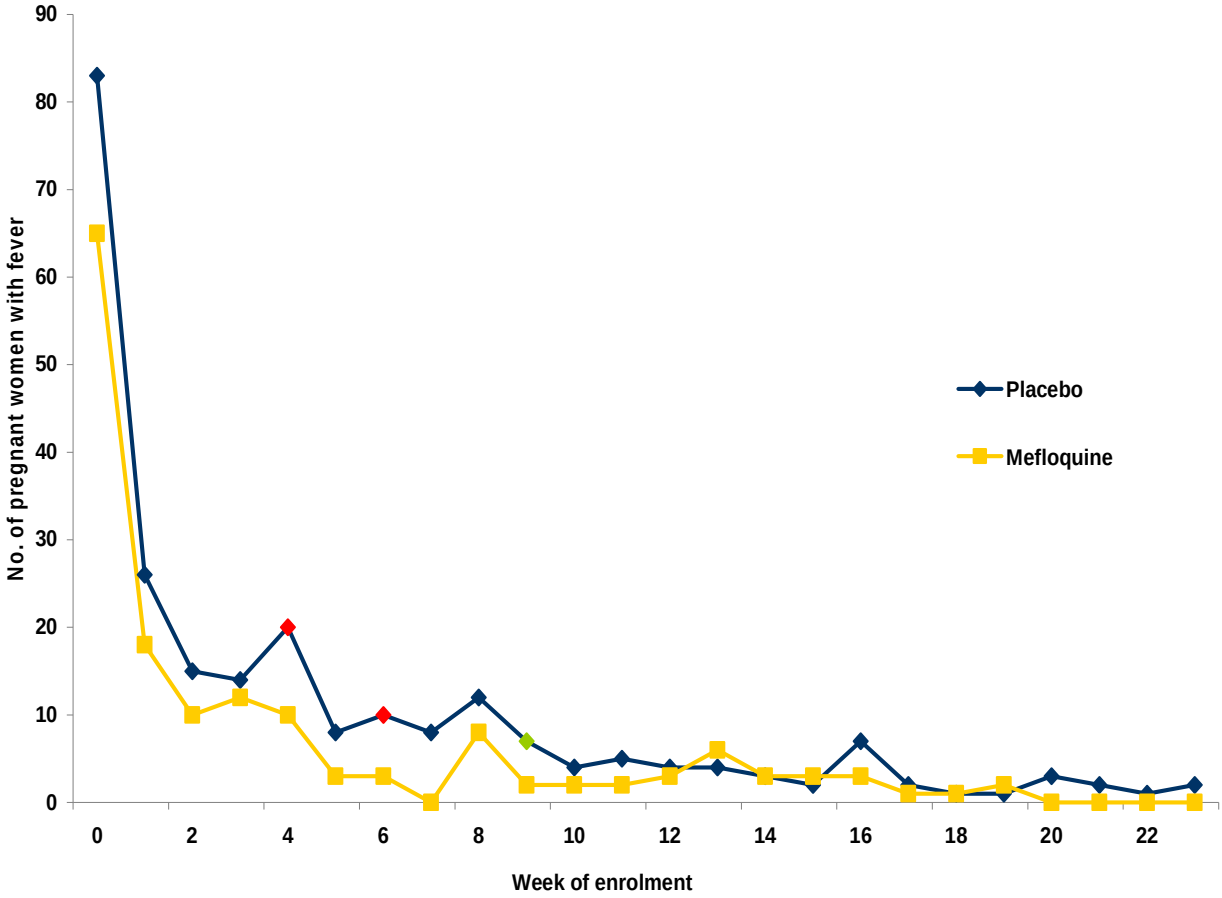



- **Purple colour indicates significant difference at p= 0.03**
- **Green colour indicates significant difference at p= 0.008**
- **Red colour indicates significant difference at p= 0.0001**

**Fig. 4.2: Incidence of parasitemia in pregnant women during chemoprophylaxis with mefloquine 250mg weekly**

### 4.3.2 The incidence of fever in pregnant women taking mefloquine prophylaxis weekly

There were significantly more reports of fever in the placebo group than in the mefloquine group. These differences were recorded in weeks 4, 6, and 7 ($p=0.05$, $p=0.05$, and $p=0.01$ respectively). The reports of fever decreased with increasing number of weeks in the study until there was almost none at the end of the study for both groups (Fig.4.3 and Appendix IX).




- **Red colour indicates significant difference at p= 0.05**
- **Light green colour indicates significant difference at p= 0.01**

**Fig.4.3: Incidence of fever in pregnant women taking mefloquine chemoprophylaxis**

### 4.3.3 Incidence of malaria parasitemia in maternal and cord blood at delivery

Thick and thin blood films were made from the maternal and cord blood at delivery. A total of 157 maternal and 159 cord blood samples of the mefloquine group and 161 and160 maternal and cord blood samples respectively of the placebo group, were examined. No malaria parasites were found in all the slides (Table 4.7).

**Table 4.7: Incidence of malaria parasitemia in maternal and cord blood at delivery**

| Study Group | | Malaria parasite negative (Mother) | Malaria parasite negative (Child) |
|---|---|---|---|
| **Mefloquine** | **Low parity** | 64(40.8) | 66(41.5) |
| | **High parity** | 93(59.2) | 93(58.5) |
| | **Total** | 157(100) | 159(100) |
| **Placebo** | **Low parity** | 57(35.4) | 55(34.4) |
| | **High parity** | 104(64.6) | 105(65.6) |
| | **Total** | 161(100) | 160(100) |

- **Numbers in parenthesis are percentages**

## 4.4 Effect of Mefloquine on outcome of pregnancy

Chemoprophylaxis against malaria for pregnant women in endemic areas is recommended to prevent the adverse consequences of malaria in pregnancy. It has been documented that regular malaria prophylaxis during pregnancy, prevents malaria attack, and associated complications like spontaneous abortions, low birth weight babies and maternal anaemia.

### 4.4.1 Haematocrit levels of pregnant women at enrolment and every ANC

Blood samples were taken for measuring the haematocrit level (PCV %) of all pregnant women at enrolment and at every ANC attended. This parameter is used to check for anaemia. Anaemia is known as one of the complications of pregnancy, and malaria in pregnancy has been shown to be a contributory factor.

At enrolment there was no statistically significant difference in haematocrit level of all groups. The prevalence of anaemia was 11.7% (Figure 4.4). There was also no significant difference in all groups after the first month of the study. The high parity mefloquine had significantly higher haematocrit levels at week 8 ($p=0.05$) and 12 ($p=0.027$). When all parities were combined, the mefloquine group still had significantly higher PCV levels than placebo at week 8 ($p=0.05$) and week 12 ($p=0.006$) these differences however were lost at week 16. Throughout the study period the low parity mefloquine group consistently had higher PCV levels than low parity placebo although this was not significant (Table 4.8).

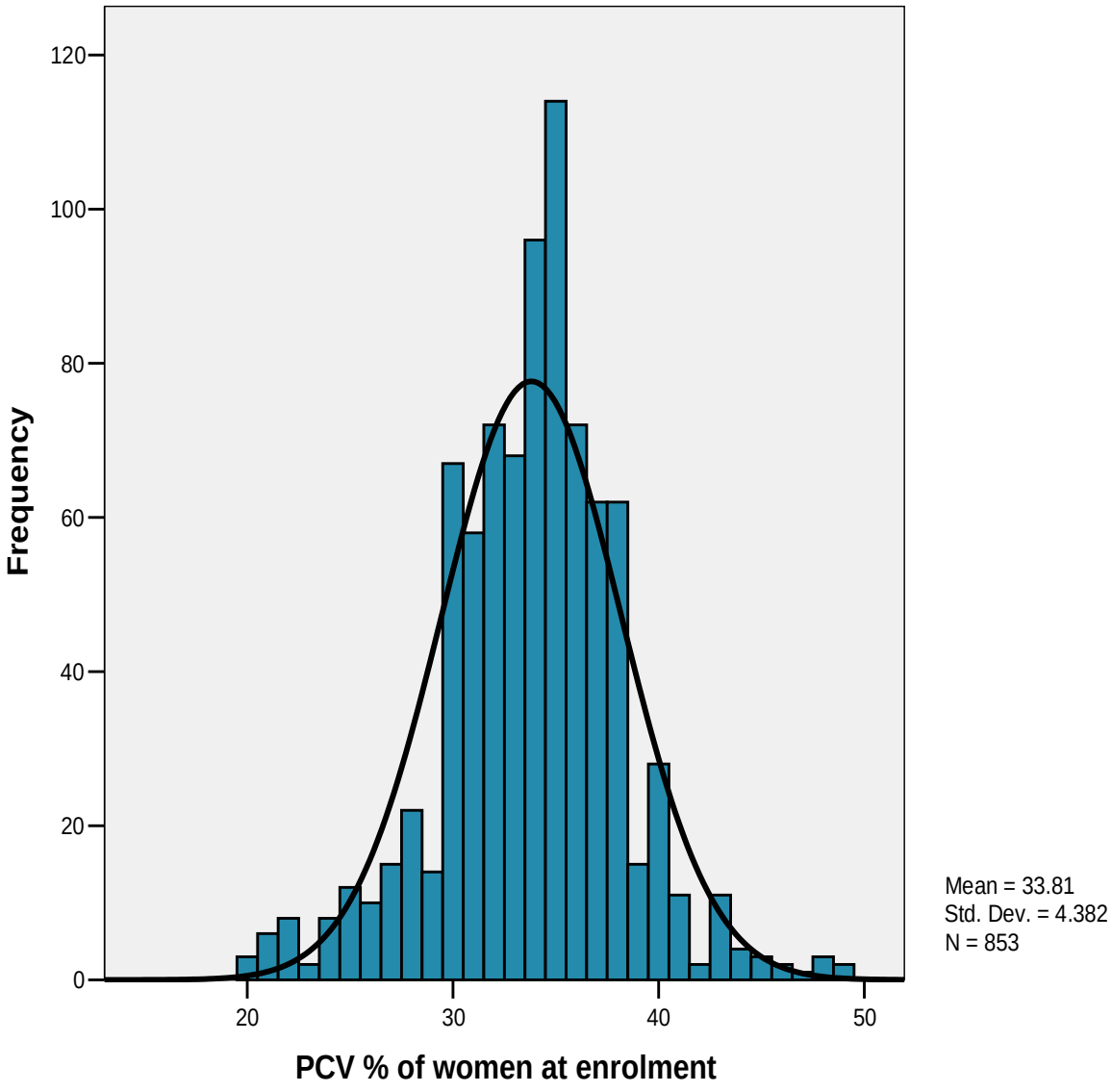



**Fig 4.4 Haematocrit level of pregnant women at enrolment**

**Table 4.8: Haematocrit level of pregnant women at enrolment and every ANC**

| Weeks of enrolment | Study group | Parity | | |
|---|---|---|---|---|
| | | Low parity | High parity | Total |
| **Day zero** | **Placebo** | 34.02±4.47(166) | 33.98±4.28(253) | 34.00±4.37(419) |
| | **Mefloquine** | 33.70±4.65(177) | 33.61±4.25(251) | 33.63±4.38(428) |
| **4** | **Placebo** | 33.52±3.36(122) | 33.27±3.52(161) | 33.35±3.44(285) |
| | **Mefloquine** | 33.18±4.27(122) | 33.30±3.44(178) | 33.28±3.80(298) |
| **8** | **Placebo** | 33.59±3.86(109) | 32.96±3.55(139) | 33.20±3.69(247) |
| | **Mefloquine** | 33.84±3.56(114) | 33.74±3.25(144)***** | 33.81±3.37(259)***** |
| **12** | **Placebo** | 33.28±3.54(92) | 32.93±3.34(144) | 33.08±3.42(207) |
| | **Mefloquine** | 34.14±3.91(103) | 34.02±3.89(105)****** | 34.08±3.89(208)******* |
| **16** | **Placebo** | 33.88±3.21(78) | 33.43±4.27(83) | 33.65±3.78(161) |
| | **Mefloquine** | 34.27±4.27(71) | 34.63±3.83(73) | 34.45±4.05(144) |

- **Data are expressed as Mean ± SD**
- **Numbers in parenthesis are sample size**

***p=0.005 **p=0.027 ***p=0.006**

### 4.4.2 Delivery Outcome

There were 586 deliveries that could be accounted for while the others were lost to follow up. The mefloquine group was 300 (51.2%) deliveries while 286(48.8%) were of the placebo group. The total foetal loss in the study was 41(7%). There were 12(4%) abortions, and 7(2.3%) stillbirths in the mefloquine group, as compared to 5(1.7%) abortions, and 17(5.9%) of stillbirths in the placebo group (Table 4.9). There were 10(1.75%) multiple births, with 5 each for both mefloquine and placebo groups. There was a statistically significant difference between the mefloquine group and the placebo group and this showed that the delivery outcome was dependent on the group ($p=0.027$). The results showed more abortions in the mefloquine group while there were more stillbirths in the placebo group.

The types and incidence of congenital malformations that occurred in the study are shown in Tables 4.10 and 4.11. The incidence of occurrence in both study groups were similar, 5 (1.67%) and 5 (1.75%) for mefloquine and placebo respectively. This makes a prevalence rate of 16.7/1000 births for the mefloquine group and 17.5/1000 for the placebo group. One of the babies in the mefloquine group had multiple malformations consisting of hydrocephalus, cleft lip and palate, and talipes of both feet. There was an occurrence of one hydrocephalus and one abnormal growth in the anal region in each group. Ventricular septal defect and a laryngeal stridor were only seen in the mefloquine group. However, two of the malformations that occurred in the placebo group were unspecified. Three out of the five babies in the placebo group born with a congenital malformation were stillbirths while one in the mefloquine group was a stillbirth.

**Table 4.9: The outcome of delivery in 586 pregnant women taking weekly mefloquine prophylaxis**

| Study Group | Live Birth | Abortion | Stillbirth | Total |
|---|---|---|---|---|
| **Mefloquine** | 281(93.7) | 12(4.0) | 7(2.3) | 300(100) |
| **Placebo** | 264(92.3) | 5(1.7) | 17(5.9) | 286(100) |
| **Total** | 545(93.0) | 17(2.9) | 24(4.1) | 586(100) |

- **Numbers in parentheses are percentages**

  **$X^2$ = 7.249, df=2, p-value = 0.027 (statistically significant)**

**Table 4.10: Incidence of Congenital malformations by Study group**

| | Congenital malformation | | |
|---|---|---|---|
| **Study group** | **Present** | **None** | **Total** |
| **Mefloquine** | 5(1.67) | 295(98.3) | 300(100) |
| **Placebo** | 5(1.75) | 281(98.25) | 286(100) |
| **Total** | 10(1.7) | 576(98.3) | 586(100) |

- **Numbers in parenthesis are percentages**

  **$X^2$=0.01, df=1, p-value=0.920 (Not statistically significant)**

**Table 4. 11: Types of Congenital Malformations Occurring in the Study**

| | Number Occurring in Study | |
|---|---|---|
| **Type of Congenital Malformation** | **Placebo** | **Mefloquine** |
| **Hydrocephalus** | 1 | 1 |
| **Club foot** | 1 | - |
| **Growth in Anal Region** | 1 | 1 |
| **Ventricular Septal Defect** | - | 1 |
| **Laryngeal Stridor** | - | 1 |
| **Multiple malformations(Hydrocephalus with Cleft palate and Club feet)** | - | 1 |
| **Not Specified** | 2 | - |

#### 4.4.2.1 *Place of Delivery*

The place of delivery was recorded, and 46% of the women in the study delivered their babies at home, while 16.4% delivered at the hospital. There was no record of delivery for 37.5%. The distribution of hospital and home deliveries between the study groups was identical with 74.4% of women in the placebo group and 73% in the mefloquine group had their babies at home (Table 4.12).

**Table 4.12: Distribution by Study group and Place of delivery**

| | Place of delivery | | |
|---|---|---|---|
| **Study group** | **Home** | **Hospital** | **Total** |
| **Mefloquine** | 230(73) | 85(30) | 315(100) |
| **Placebo** | 224(74.4) | 77(25.6) | 301(100) |
| **Total** | 454(73.7) | 162(26.3) | 616(100) |

- **Numbers in parenthesis are percentages**

### 4.4.3 Effect of Mefloquine chemoprophylaxis on anthropometry of babies born to women in the study

The birth weight, birth length, mid-arm circumference (MAC) and Occipito-frontal head circumference (OFC) were measured for all babies born in the hospital within the first 24 hour as of birth (Table 4.13). Some of the babies that were born at home were brought to the hospital within the first 48 hours and these measurements were taken while others were lost to follow up.

Birth weight of babies born to women in the mefloquine and placebo groups did not show any significant difference when both low and high parity groups were combined in each group. When the groups were subdivided into low and high parity, there was a significant difference between the mean birth weights of babies born to women of high parity. There was a difference of 110gm ± 0.054 with the mefloquine group having heavier babies. The low parity group however, did not show any significant difference between the two groups as shown in Table 4.13.

The frequency of low birth weight (LBW) in the two study groups was similar (10%) (Table 4.14). Comparing the birth weights of LBW babies in the low parity groups shows a significant difference with the placebo group being heavier ($p=0.015$) by 178±15g (Table 4.15).

**Table 4.13: Anthropometric measurements of babies of women in the study groups**

| Study Group | | Birth weight (kg) | Birth length(cm) | Mid-arm circumference (cm) | Occipito-frontal circumference (cm) |
|---|---|---|---|---|---|
| **Mefloquine** | **Low parity** | 2.85±0.49(93) | 47.45±3.72(92) | 10.77±0.93(94) | 33.76±1.69(94) |
| | **High parity** | 3.01±0.37(130)***** | 47.83±3.71(126) | 11.11±1.02(127) | 34.22±1.69(126)***** |
| | **Total** | 2.94±0.45(223) | 47.67±3.70(218) | 10.96±0.99(221) | 34.02±1.70(220) |
| **Placebo** | **Low parity** | 2.83±0.40(91) | 47.35±3.46(88) | 10.84±0.96(88) | 33.52±1.86(88) |
| | **High parity** | 2.90±0.39(132) | 47.63±3.97(129) | 10.85±1.18(128) | 33.73±1.91(94) |
| | **Total** | 2.89±0.40(221) | 47.59±3.70(217) | 10.87±1.06(214) | 33.71±1.76(214) |

- **Data are express as Mean ± SD**
- **Numbers in parenthesis are sample size**
- ***p-value <0.05**

**Table 4.14: Frequency distribution of birth weight of babies born to women on mefloquine chemoprophylaxis**

| | *Placebo* | | | *Mefloquine* | | |
|---|---|---|---|---|---|---|
| ***Birth Weight(Kg)*** | ***Low Parity*** | ***High Parity*** | ***Total*** | ***Low Parity*** | ***High Parity*** | ***Total*** |
| **1.00-1.49** | | | | 1(1.1) | | **1(0.4)** |
| **1.50-1.99** | | 2 (1.5) | **2(0.9)** | 3(3.2) | 1(0.8) | **4(1.8)** |
| **2.00-2.49** | 13(14.3) | 7(5.3) | **20(9.0)** | 12(12.9) | 7(5.4) | **19(8.5)** |
| **2.50-2.99** | 46(50.5) | 64(49.2) | **110(49.8)** | 34(36.6) | 48(36.9) | **82(36.8)** |
| **3.00-3.49** | 24(26.4) | 43(33.7) | **67(30.3)** | 32(34.4) | 55(42.3) | **87(39.0)** |
| **3.50-3.99** | 7(7.7) | 13(10.0) | **20(9.5)** | 11(11.8) | 15(11.5) | **26(11.7)** |
| **4.00-4.49** | 1(1.1) | 1(0.8) | **2(0.9)** | | 4(3.1) | **4(1.8)** |
| **Total** | 91(100) | 130(100) | **221(100)** | 93(100) | 130(100) | **223(100)** |

- **Numbers in parenthesis are percentages**

**Table 4.15: Mean birth weight for Low Birth Weight babies born to women on mefloquine prophylaxis**

| Study Group | Parity | | |
|---|---|---|---|
| | **Low Parity** | **High Parity** | **Total** |
| **Placebo** | 2.28±0.16(13)***** | 2.21±0.24(9) | 2.25±0.19(22) |
| **Mefloquine** | 2.09±0.20(16) | 2.30±0.15(8) | 2.17±0.21(24) |

*** p=0.015**

- **Data are expressed as Mean±SD**
- **Numbers in parenthesis are sample size**

#### 4.4.3.1 *The effect of sex of baby on Mean birth weight*

In order to see the effect of sex of the baby on birth weight, the birth weight of males were compared to that of females. Males were 46.7% of the total babies born to women in the study and they were equally distributed in both study groups. The mean birth weight for both sexes is shown in Table 4.16. In the low parity placebo group the male babies were significantly heavier (p=0.029) than females which is not seen when the two sexes were compared in the low parity mefloquine group (p=0.836). Female babies in the high parity mefloquine group were also significantly heavier than females in the high parity placebo group (p=0.022). When all parities were combined, females in the mefloquine group were significantly heavier than females in the placebo group (p=0.011) but when the males were compared, there was none (p=0.861).

**Table 4.16: The effect of sex of baby on Mean birth weight (Kg)**

| Sex of baby | Mefloquine | | Placebo | |
|---|---|---|---|---|
| | Low Parity | High Parity | Low parity | High parity |
| **Male** | 2.82±0.57 (32) | 2.99±0.41 (58) | 2.93±0.42 (37) | 2.95±0.54 (52) |
| **Female** | 2.85±0.46 (47) | 2.99±0.47 (55) | 2.73±0.36 (36) | 2.79±0.48 (66) |

- **Data are expressed as Mean ± SD**
- **Numbers in parenthesis are sample size**

### 4.4.4 Placental weight and histopathology

#### 4.4.4.1 *Mean weight of placenta (gm) in the study*

The mean placental weight (gm) ± SD of 143 placentas in the mefloquine group was 483.2±111.7 and there was no significant difference 475.3 ± 108.5 of 149 placentas of the placebo group. When the study groups were subdivided into parities, there was no significant difference even though the mefloquine group had consistently heavier placentas (Table 4.17).

**Table 4.17: Mean weight of placenta (gm) in the study group**

| Study Group | | Mean ±SD |
|---|---|---|
| **Mefloquine** | **Low parity** | 476.0±109.9 |
| | | **(56)** |
| | **High parity** | 487.9±113.3 |
| | | **(87)** |
| | **Total** | 483.2±111.7 |
| | | **(143)** |
| **Placebo** | **Low parity** | 459.9±100.8 |
| | | **(53)** |
| | **High parity** | 483.8±112.1 |
| | | **(96)** |
| | **Total** | 475.3±108.5 |
| | | **(149)** |

- **Data are expressed as Mean±SD**
- **Numbers in parenthesis are sample size**

#### 4.4.4.2 *Histopathology of Placenta*

A total of 52 placenta samples were studied for histopathology changes, out of which 21 (40%) were from women in the placebo group and 31 (60%) were from the mefloquine group. When the samples from both groups were further subdivided, 13 and 4 belonged to low parity mefloquine and placebo groups respectively. The mefloquine high parity group had 18 samples and placebo high parity were 17. The high parity groups were associated with a high incidence of calcium, fibrin and malaria pigment deposition. They were also associated with the presence of malarial parasite in the placental tissues. The sample size of the placentas available for histopathological studies was 5.2% of the total study population. This is not representative of the study population and the samples were skewed towards the high parity group.

***Incidence of Calcium Deposition***

Calcium Deposition was found in 75% of low parity and 19% high parity placebo groups, while it was found in 38.5% and 50% of low and high parity mefloquine group samples respectively. (Table 4.18)

***Incidence of Malaria Pigmentation***

Malarial pigments were found to have been deposited in most of the samples that were studied, 81% and 93.6% of placebo and mefloquine groups respectively. This shows both past and active malaria infections (Table 4.19).

### *Incidence of Fibrin Deposition*

Fibrin deposits were found in almost all the placenta. All the samples of the low parity groups in both study groups had fibrin deposits. The high parity groups had 76.5% and 83.5% incidence of fibrin deposition for placebo and mefloquine groups respectively (Table 4.20).

### *Incidence of Malaria parasite*

The placentas were found to have low malaria infestation. The low parity mefloquine group only showed 38.5%; while the low parity placebo group had 50%. This shows that the samples had malaria parasites and about half of the high parity groups of both study groups had malaria parasites (Table 4.21).

**Table 4.18: Presence of calcium deposits in placenta of women in the study**

| Study group | | Placental Pathology - Calcium | | | |
|---|---|---|---|---|---|
| | | **Negative** | **1+** | **2+** | **3+** |
| **Placebo** | **Low parity** (4) | 1 | 1 | 2 | 0 |
| | **High parity** (17) | 13 | 2 | 1 | 1 |
| **Mefloquine** | **Low parity** (13) | 8 | 4 | 1 | 0 |
| | **High parity** (18) | 9 | 6 | 3 | 0 |

- **Numbers in parenthesis are sample size**

**Key**
**1+ = Mild deposition**
**2+ = Moderate deposition**
**3+ = Heavy deposition**

**Table 4.19: Presence of malaria pigments in placenta of women in the study**

| Study group | | Placental Pathology – Pigment | | |
|---|---|---|---|---|
| | | **Negative** | **1+** | **2+** |
| **Placebo** | | | | |
| | **Low Parity** (4) | 0 | 2 | 2 |
| | **High parity** (17) | 4 | 10 | 3 |
| **Mefloquine** | | | | |
| | **Low Parity** (13) | 1 | 12 | 0 |
| | **High Parity** (18) | 1 | 13 | 4 |

- **Numbers in parenthesis are sample size**

**Key**
**1+ = Mild deposition**
**2+ = Moderate deposition**

**Table 4 .20: Incidence of fibrin deposition in placenta of women in the study**

| Study group | Placental Pathology - Fibrin | | | |
|---|---|---|---|---|
| | **Negative** | **1+** | **2+** | **3+** |
| **Placebo** | 0 | 3 | 1 | 0 |
| **Low Parity** (4) | | | | |
| **High parity** (17) | 4 | 9 | 3 | 1 |
| **Mefloquine** | 1 | 6 | 6 | 0 |
| **Low Parity** (13) | | | | |
| **High Parity** (18) | 3 | 6 | 8 | 1 |

- **Numbers in parenthesis are sample size**

**Key**
**1+ = Mild deposition**
**2+ = Moderate deposition**
**3+ = Heavy deposition**

**Table 4.21: Presence of malaria parasites in placenta of women in the study**

| Study group | | Placental Pathology – MPS | | |
|---|---|---|---|---|
| | | **Negative** | **1+** | **2+** |
| **Placebo** | | | | |
| | **Low Parity** (4) | 2 | 2 | 0 |
| | **High parity** (17) | 9 | 8 | 0 |
| **Mefloquine** | | | | |
| | **Low Parity** (13) | 7 | 5 | 0 |
| | **High Parity** (18) | 9 | 6 | 3 |

- **Numbers in parenthesis are sample size**

**Key**
**1+ = Mild infestation**
**2+ = Moderate infestation**

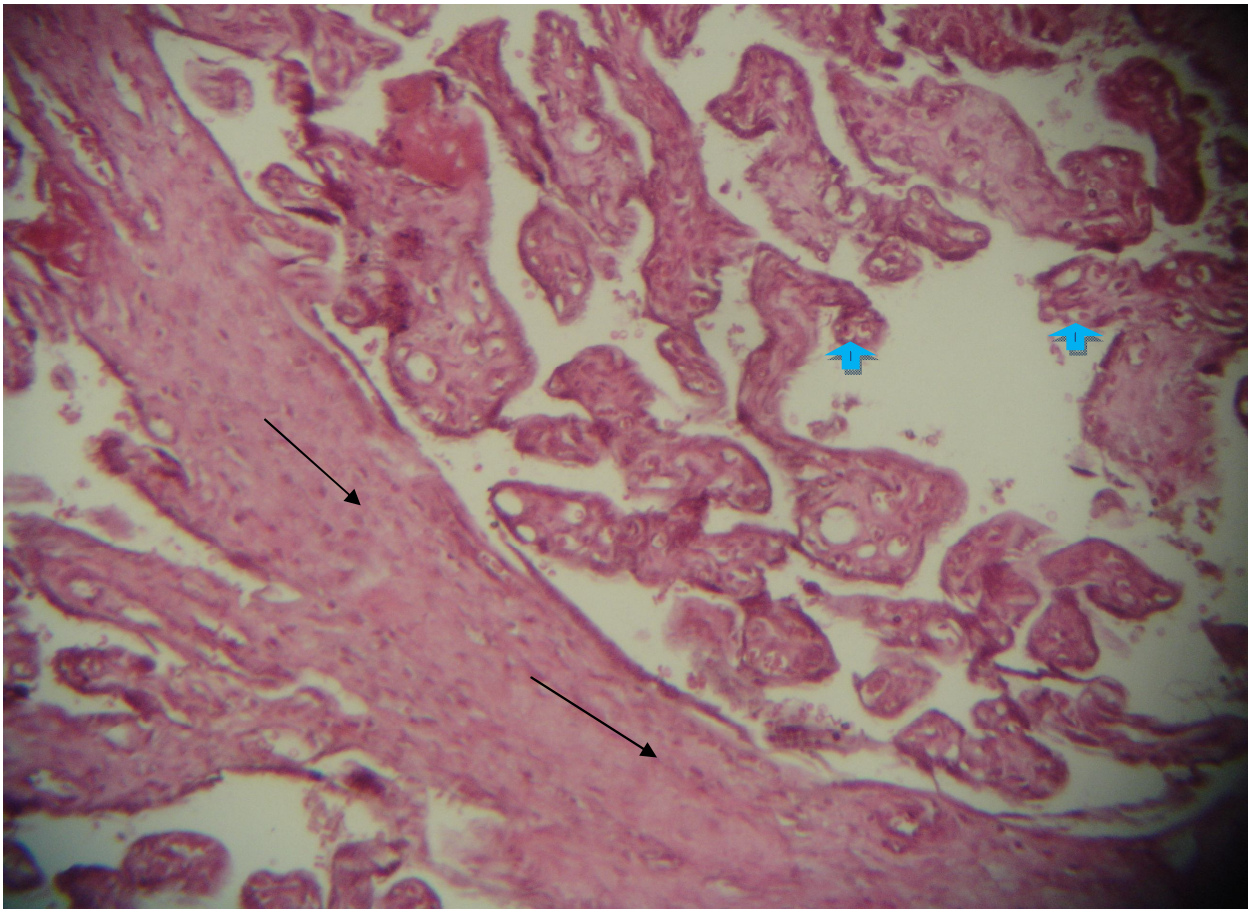


**Plate 4.1: Photomicrograph of normal placenta showing foetal membrane (⟶) and chorionic villi (⬆) (H & E x 360)**

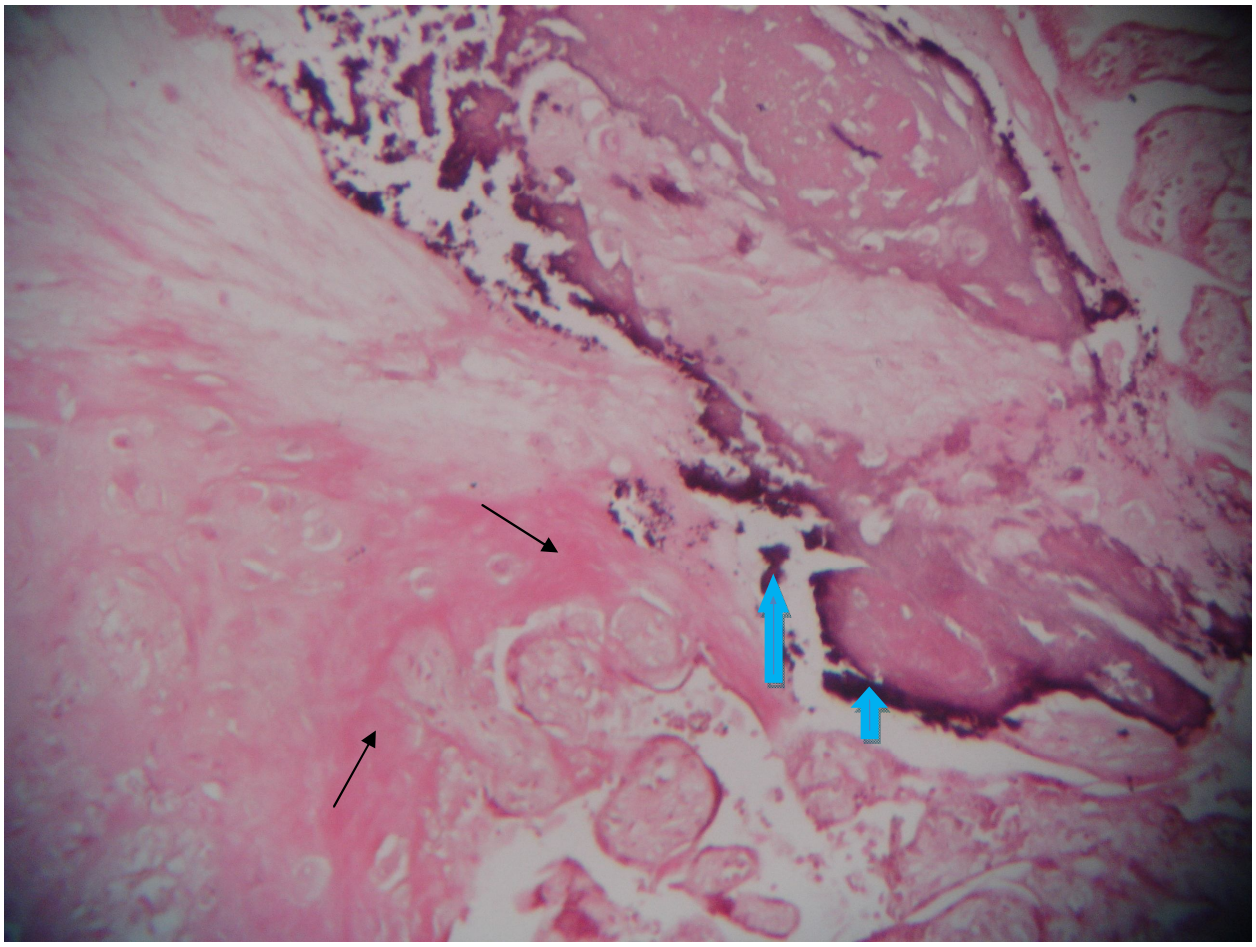


**Plate 4.2: Photomicrograph of placenta with fibrin deposit (⟶) and Calcification (in black granules) ( ⟹ ) (H & E x 180)**

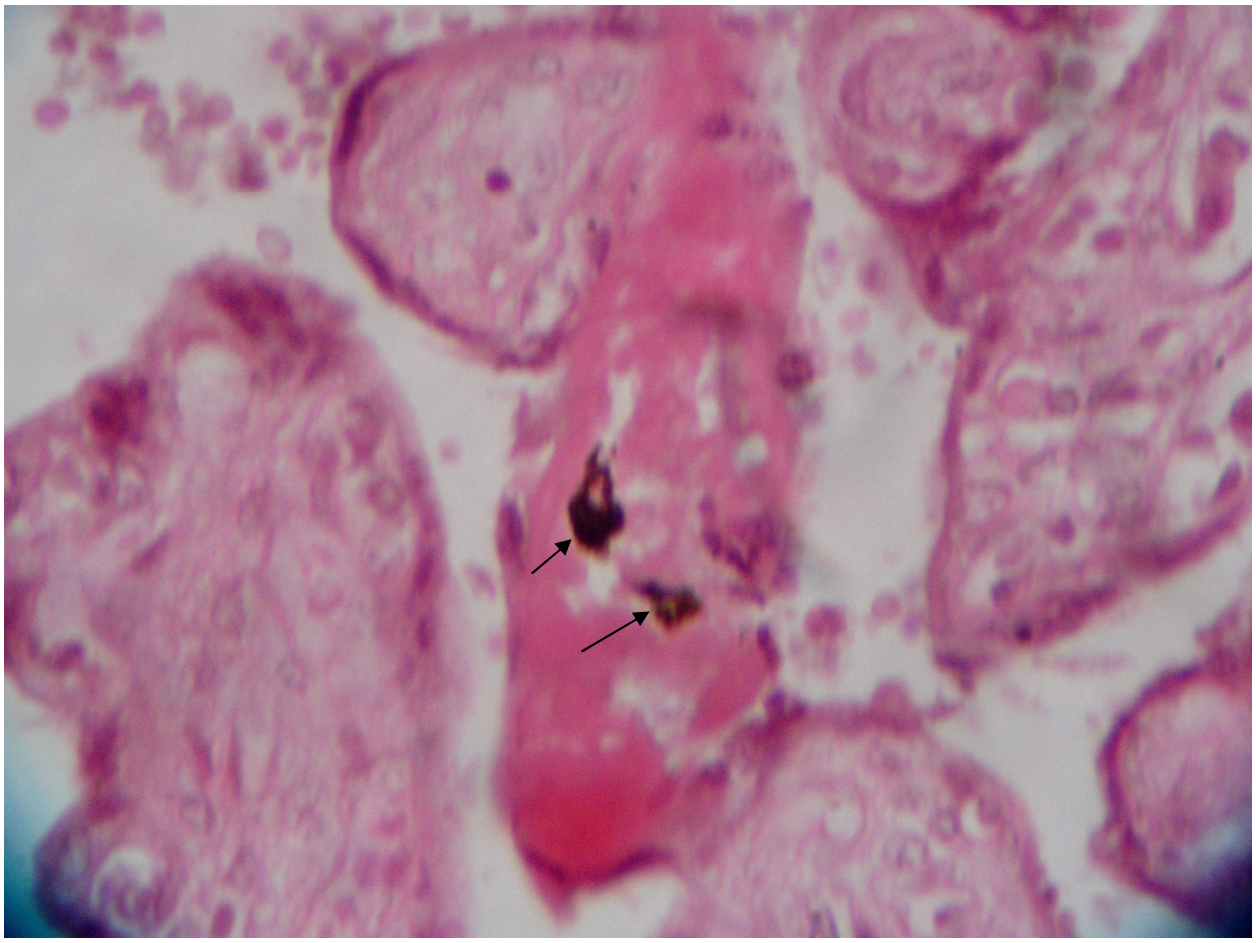


**Plate 4.3: Photomicrograph of Placenta showing Malarial pigment ( ⟶ ) in Perivillous Fibrin (H & E x 360)**

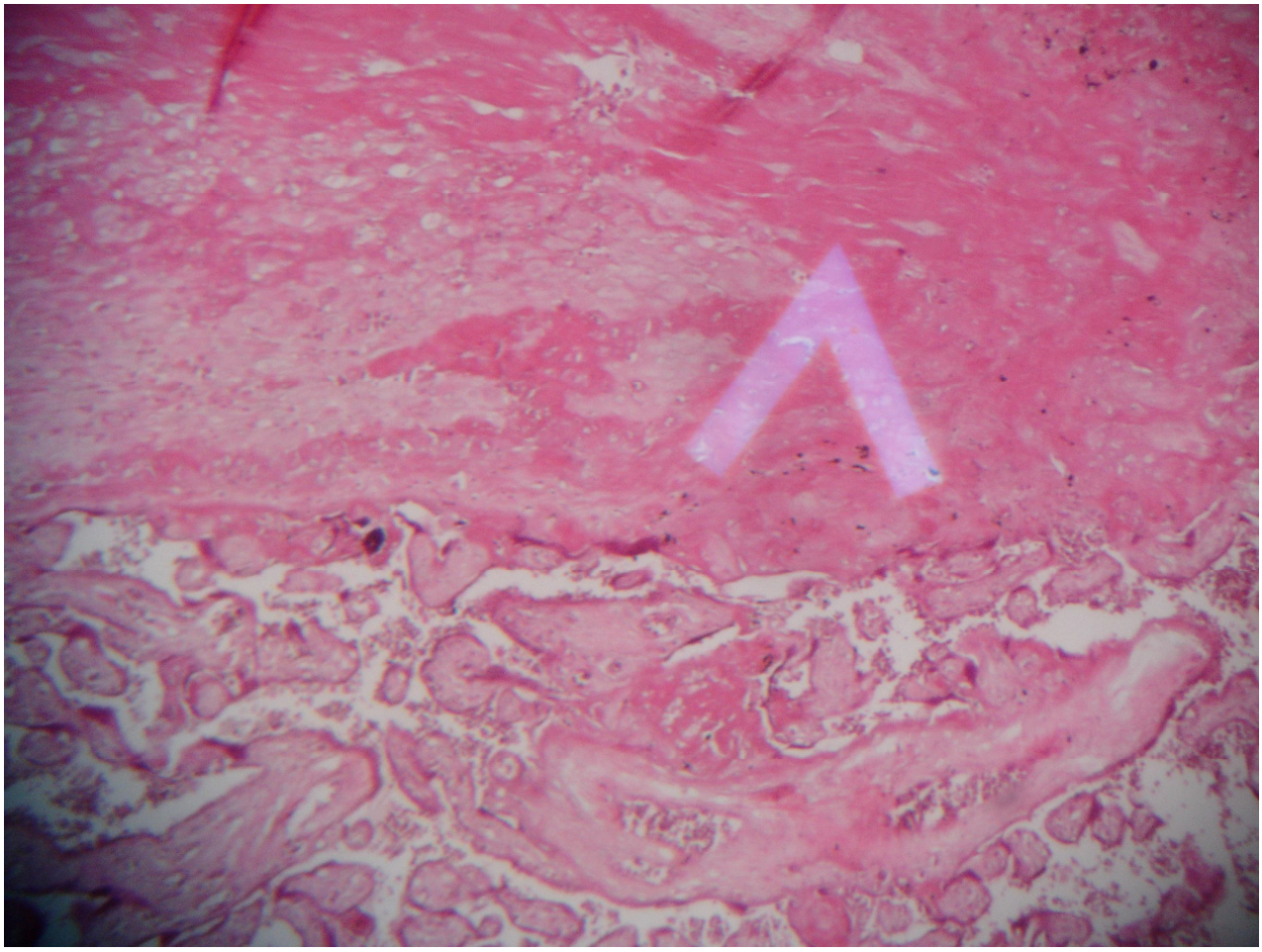


**Plate 4.4: Photomicrograph of placenta showing Extensive Fibrin Deposition (in pink) (H & E x 360)**

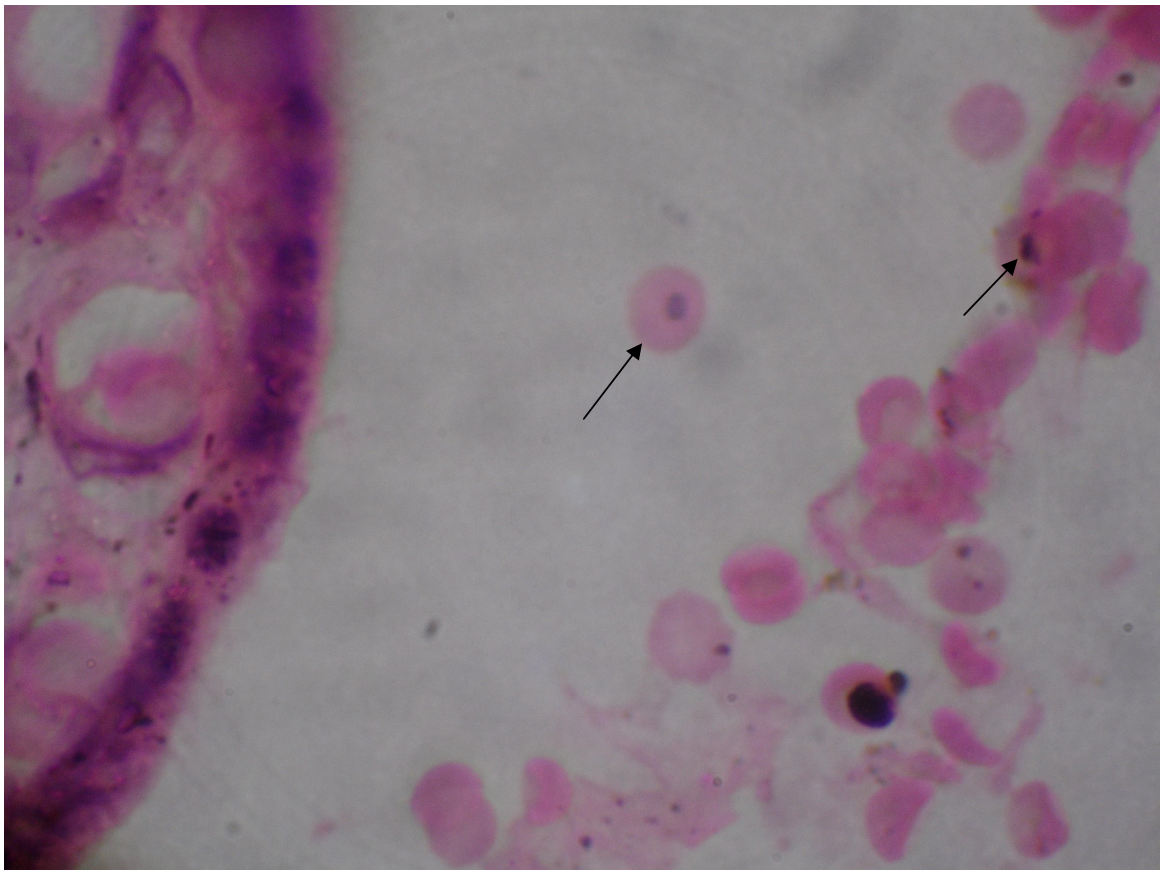


**Plate 4.5: Photomicrograph of placenta showing malaria parasite in RBC ( ⟶ )**

**(H & E x 1000)**

## 4.5 Side Effects of Mefloquine in Pregnant Women

Mefloquine has been shown to be well tolerated when taken as a prophylactic by both pregnant and non-pregnant women. The most common adverse effects reported are diarrhoea, nausea, vomiting, dizziness and headache. The adverse effects that were studied were nausea, vomiting, diarrhoea, dizziness, and pruritus.

### 4.5.1 Incidence of nausea in pregnant women who are taking mefloquine (250mg), weekly

The frequency of nausea that was reported by pregnant women in both the placebo and mefloquine group was recorded weekly. There was no significant difference between the two groups, from the day of enrolment to delivery except at week four (4) where the mefloquine group reported a higher incidence of nausea ($p= 0.04$). The result showed that although there were reported cases of nausea in both groups, this was transient and was almost non-existent by the end of pregnancy. This is shown in Fig.4.5 and Appendix X.

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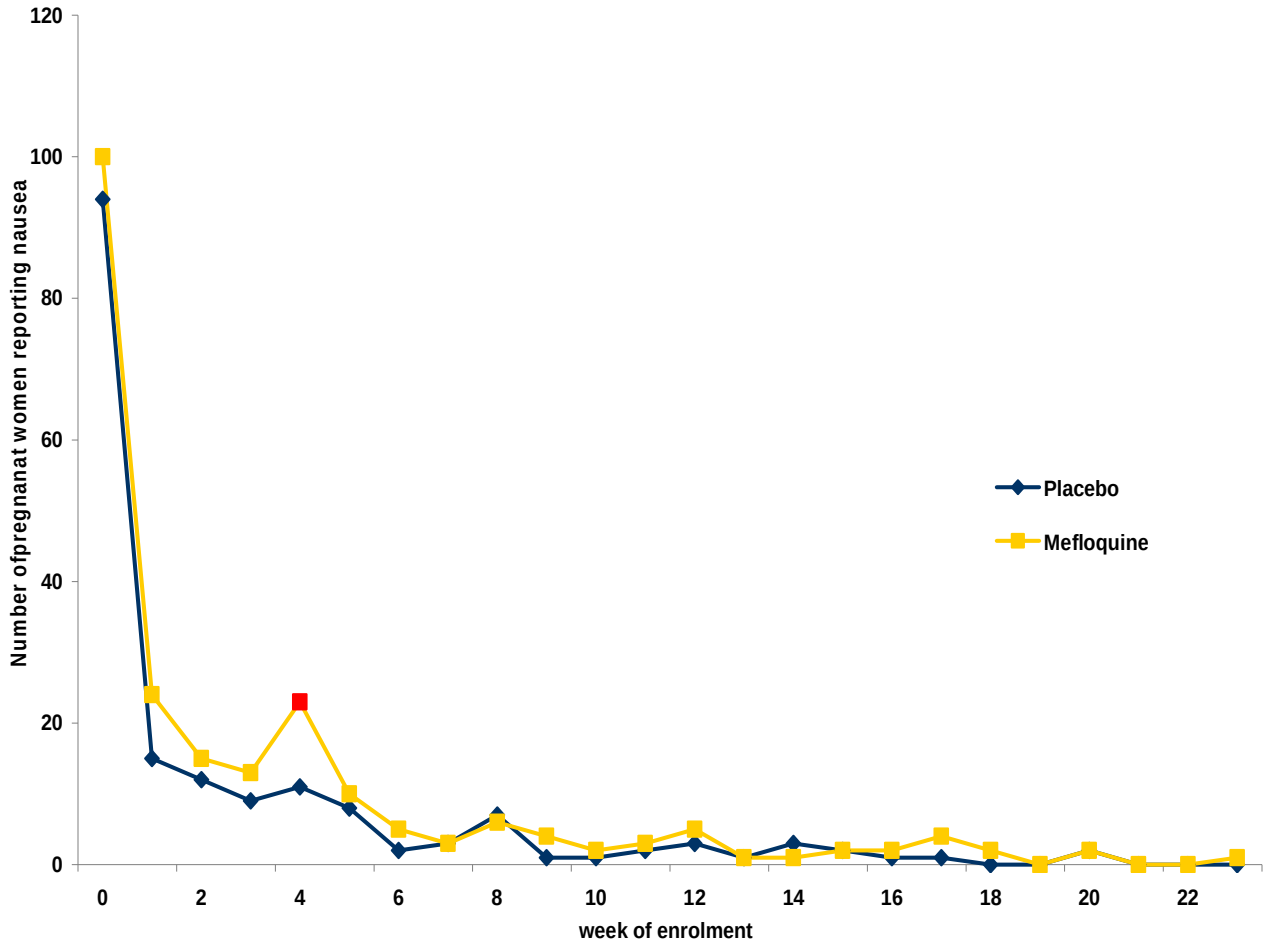


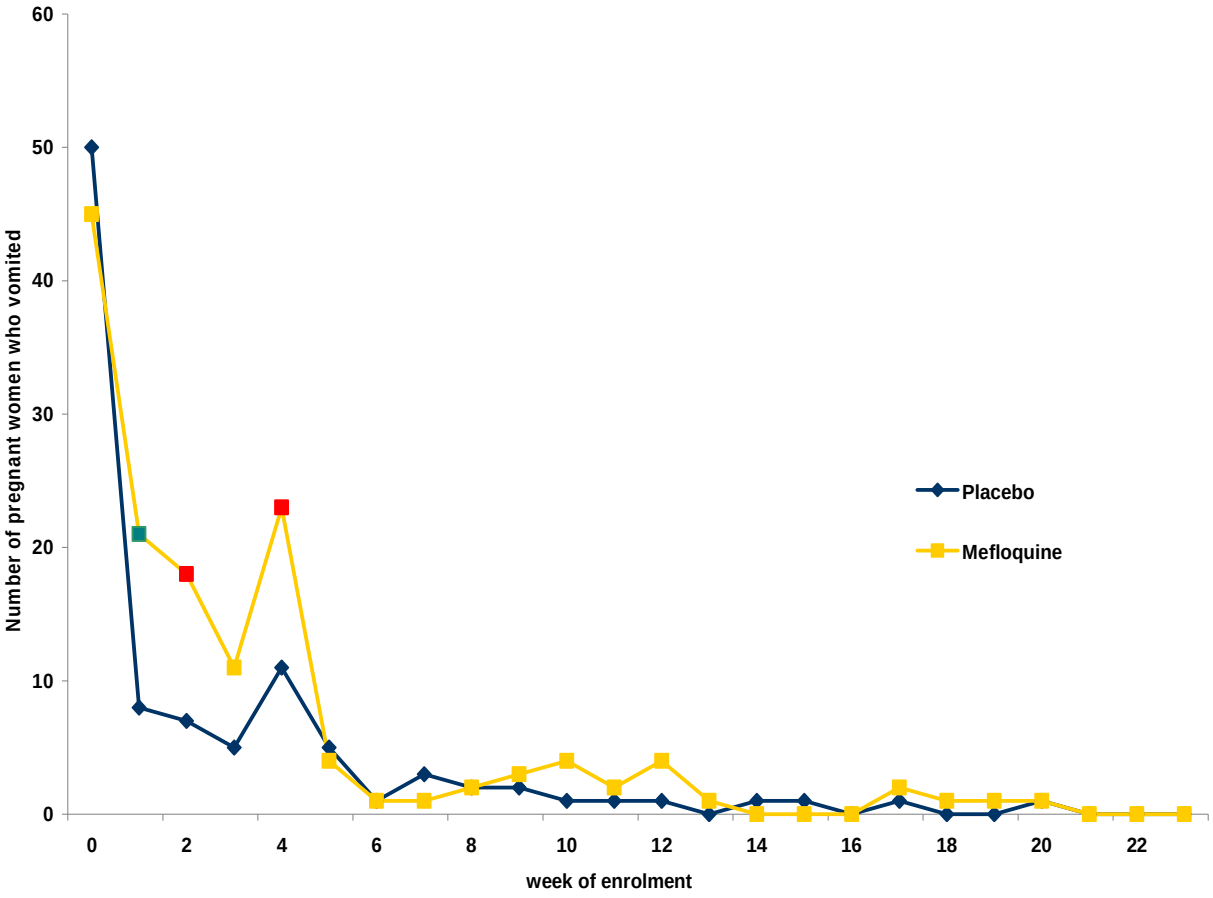

- Red colour indicates significant difference at p= 0.04

**Fig. 4.5: Incidence of nausea in pregnant women taking mefloquine (250mg) weekly**

### 4.5.2 Incidence of Vomiting in pregnant women taking mefloquine (250mg), weekly.

Vomiting is one of the most commonly reported adverse effects of mefloquine intake. In this study, one of the pregnant women enrolled was withdrawn by her husband because of profuse vomiting after taking three tablets (750mg) of mefloquine on the day of enrolment. We were unable to confirm the cause of the vomiting but the woman attributed it to mefloquine intake.

This study showed an increase in the incidence of vomiting in the mefloquine group in the first ($p=0.02$), second ($p=0.04$), and fourth ($p=0.04$) weeks of enrolment. Subsequently, although the mefloquine group consistently had a higher incidence of vomiting it did not achieve statistical significance. The frequency reduced as the weeks increased until the end where there was no report of vomiting. This is shown in Fig.4.6 and Appendix XI.




- **Green colour indicates significant difference at p= 0.02**
- **Red colour indicates significant difference at p= 0.04**

**Fig. 4.6: Incidence of vomiting in pregnant women taking mefloquine (250mg), weekly**

### 4.5.3 Incidence of Diarrhoea reported by pregnant women taking mefloquine (250mg) weekly

Diarrhoea is also a commonly reported adverse effect of mefloquine. In this study however, there was no difference in the incidence of diarrhoea in the placebo and mefloquine groups as shown in Fig.4.7 and Appendix XII.

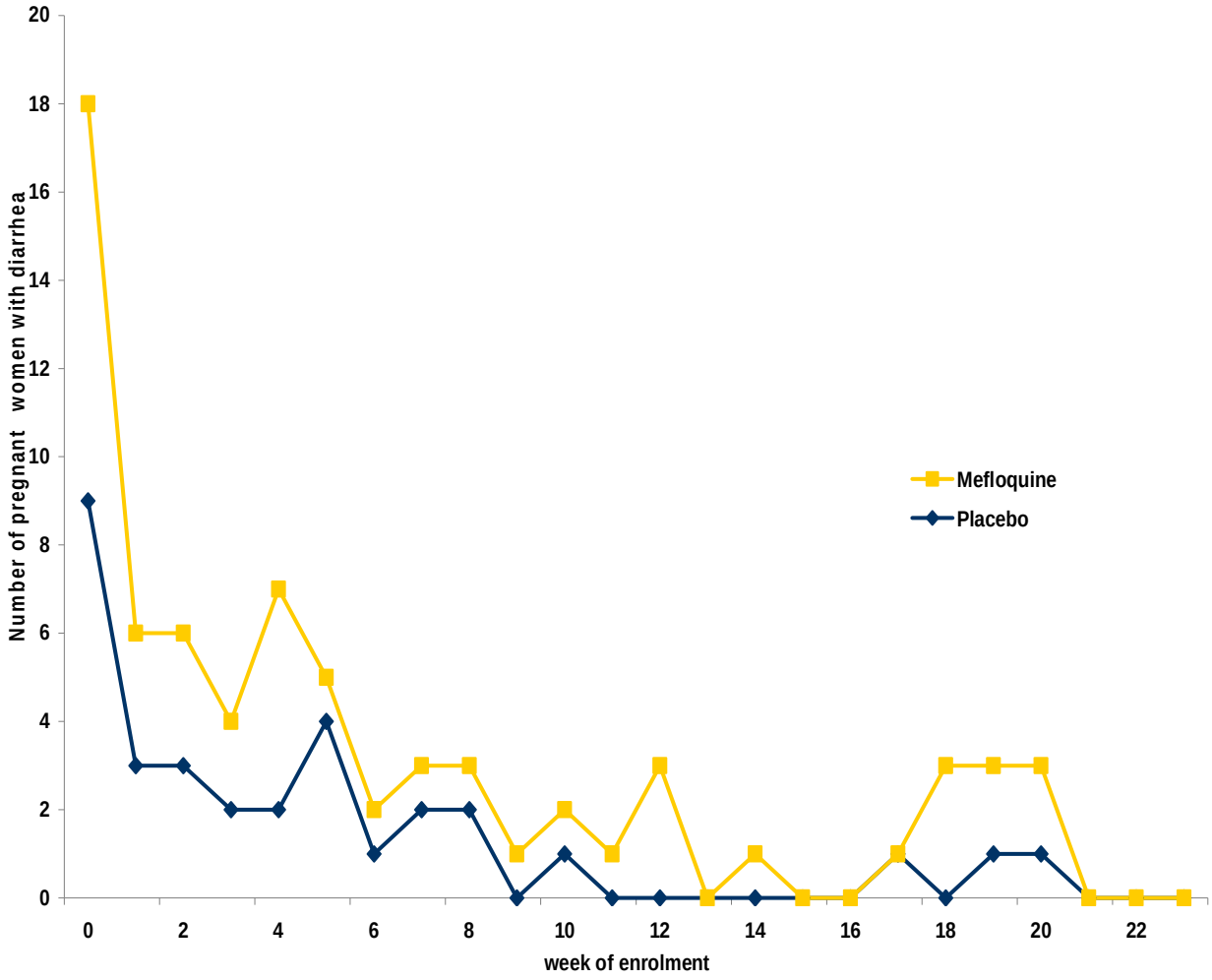
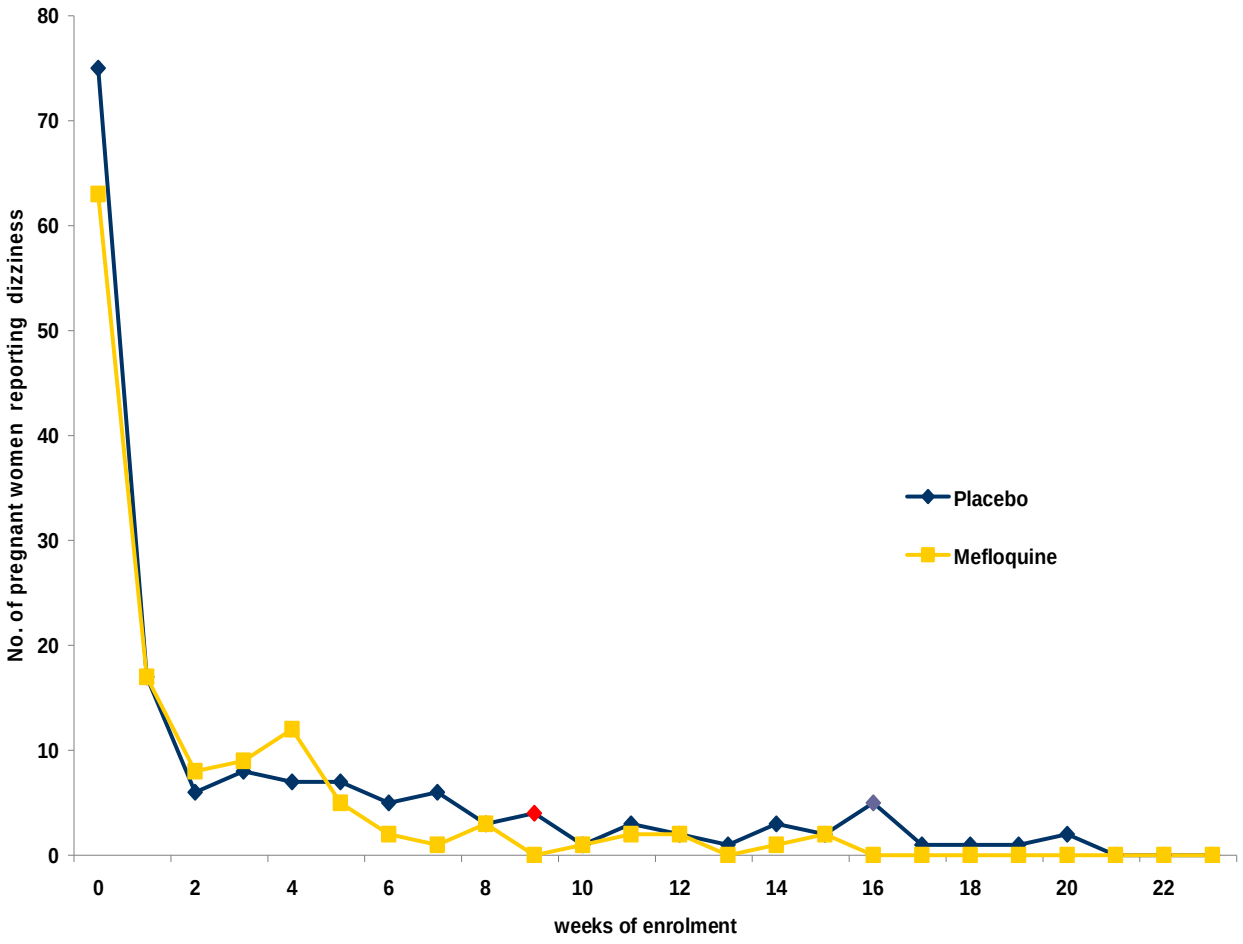



**Fig. 4.7: Incidence of diarrhoea in pregnant women taking mefloquine (250mg), weekly**

### 4.5.4 Incidence of dizziness reported by pregnant women taking mefloquine (250mg) weekly

Dizziness is one of the commonly reported side-effects of mefloquine, but it is also common in pregnancy. The incidence of dizziness was found to be significantly higher in the placebo group at week 9 and week 16 ($p=0.049$ and ($p=0.03$). The reports of dizziness decreased until there were none by week 23, as shown in Fig. 4.8 and Appendix XIII.




- **Red colour indicates significant difference at p= 0.049**
- **Purple colour indicates significant difference at p= 0.03**

**Fig. 4.8: Incidence of dizziness in pregnant women taking mefloquine (250mg), weekly**

### 4.5.5 Incidence of pruritus reported by pregnant women taking mefloquine (250mg) weekly

Pruritus was reported by women in both study group groups, mostly in the first ten weeks of enrolment and incidence decreased until by week 23. There was no significant difference between the two (2) groups (Fig. 4.9 and Appendix XIV).

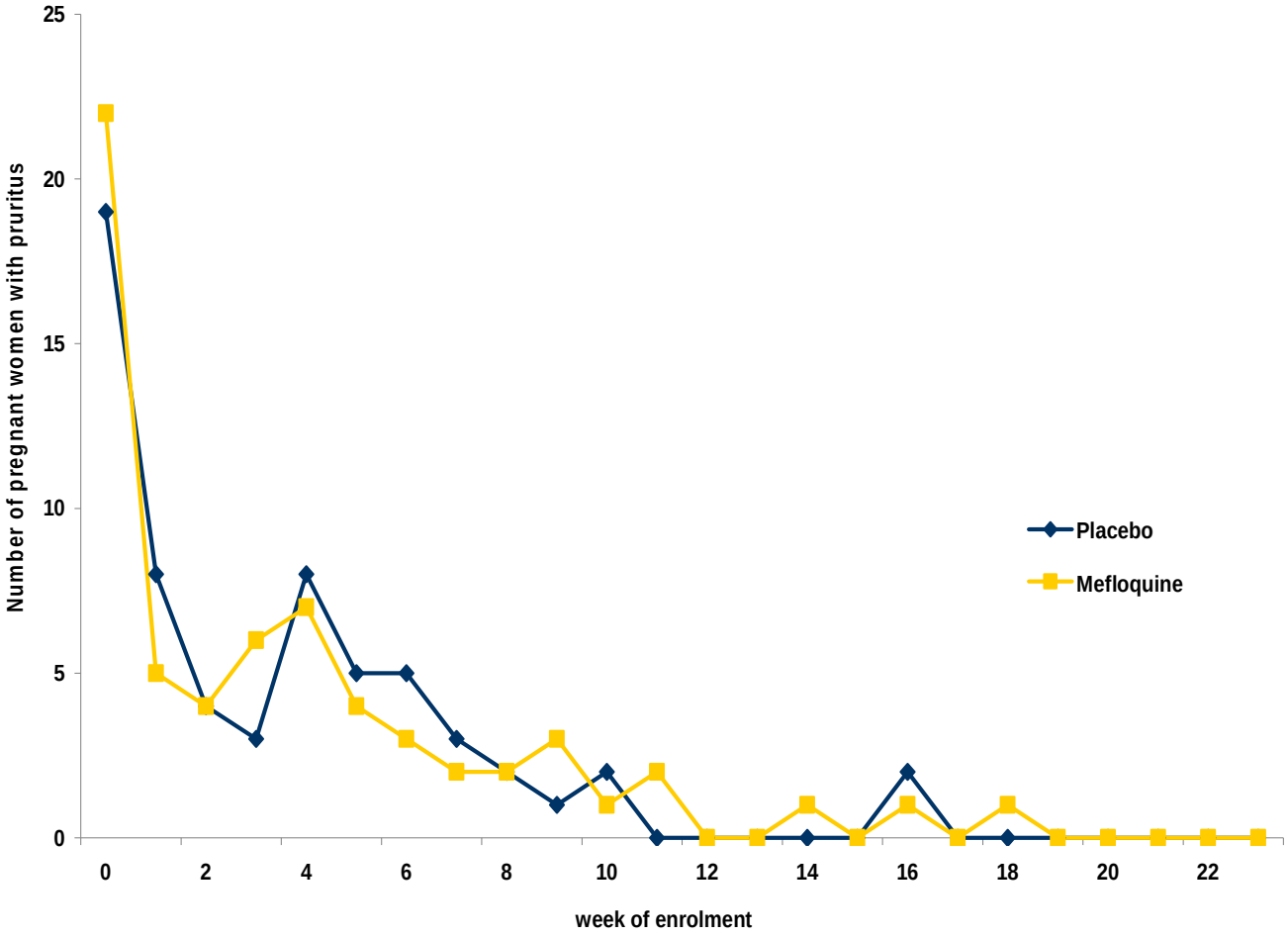



**Fig. 4.9: Incidence of pruritus in pregnant women taking mefloquine (250mg), weekly**

# CHAPTER FIVE

# DISCUSSION

Birth weight is known as one of the most important determinants of infant survival especially in tropical developing countries (Greenwood *et al.,* 1992) with low birth weight reducing child survival (Nosten *et al.,* 1994). Malaria accounts for more than 40% of low birth weight in malaria endemic areas (Brabin and Piper, 1997). In areas where falciparum malaria is endemic, most adult women have developed sufficient immunity so that even during pregnancy, *P. falciparum* infection does not usually result in fever or other clinical symptoms. In these areas, the major impact of malaria infection is maternal anaemia and the presence of malaria parasites in the placenta. These two factors contribute to low birth weight which is the greatest risk factor for neonatal and infant mortality. It is to protect against these consequences in pregnant women and their infants that antimalarial prophylaxis is recommended for pregnant women in endemic areas (WHO, 1984). However, the emergence of resistance to pyrimethamine and chloroquine necessitated the need for alternative protective measures. The WHO currently recommends intermittent preventive treatment (IPT) in pregnancy with sulfadoxine-pyrimethamine (SP), insecticide-treated bed nets, prompt case management of clinical malaria, and diagnosis and treatment of anaemia (WHO, 2000). In 2001, the Federal Ministry of Health in Nigeria adopted the WHO recommendation of IPT using two doses of SP, to be given during the second, and early in the third trimester of pregnancy (FMOH, 2005). Drug therapeutic efficacy trials conducted on chloroquine and SP in the six geopolitical zones of Nigeria, showed a high level of resistance to both drugs, which led to a change in the National malaria treatment policy (FMOH, 2005).

It was therefore necessary to study other antimalarials that can be used for prevention of malaria in pregnant women.

The results of this study have shown that mefloquine prophylaxis protected pregnant women against malaria, and was well tolerated. Malaria in association with other factors, such as nutritional status, duration of pregnancy, anaemia due to helmintic infections, HIV infection etc are known to affect birth weight. Many studies have shown the benefit of malaria chemoprophylaxis in primigravidae and secundigravidae (Nosten *et al.*, 1994). Most of these studies used control groups that did not take any form of malarial treatment. In this study, even though the placebo group did not receive any malaria prophylaxis, all positive parasitemia cases were promptly treated with a full course of chloroquine which was an effective treatment for malaria at the time the study was conducted. The women did not have to exhibit or experience the symptoms of malaria before they were treated. This could have contributed to the lack of difference in the low parity group.

The effect on birth weight was only significant in the high parity group. There was also a significant difference in birth weight between babies born to women of low and high parity within the mefloquine group, with the high parity group having heavier babies. This difference was however not seen in the placebo group. In the low parity group, no significant increase in birth weight was seen. A study in western Uganda (Ndyomugyenyi, 2000) where primigravidae were given chloroquine prophylaxis also failed to show any difference between that group and case management of malaria attacks. Another study in Burkina Faso using chloroquine prophylaxis also failed to show

an increase in birth weight in both primigravidae and all pregnant women in the study (Cot *et al.,* 1992). However in 1995, Cot *et al* demonstrated an increased mean birth weight in primigravidae who received chloroquine prophylaxis under regular supervision compared with control in Cameroon, even though there was a moderate degree of chloroquine resistance in the area. Nosten *et al*. (1994), evaluated mefloquine as a prophylactic for malaria during the second half of pregnancy in a Thai-Burmese border refugee camp. The area is mesoendemic for malaria and multi-drug resistant falciparum malaria is the most important medical problem. Mefloquine prophylaxis although proved to be safe and effective in preventing malaria illness, failed to demonstrate any significant difference in birth weight when compared to either overall or when controlled for gravidity. However, a difference was noted in the mean birth weights of babies born to women infected with *P. falciparum* compared with non-infected women in their first, second, or third pregnancies but this effect on birth weight was not seen in women of higher gravidae.

The estimated low birth weight (LBW) infants for Nigeria are 16% of live births as at 1990 (WHO, 1992b). The incidence of LBW in this study was the same in both the mefloquine and placebo groups (11 %). Babies that were born premature (gestation less than 37 weeks) and twins were excluded. There were more low birth weight babies born to women of low parity than high parity in the both groups. Although this study did not change the frequency of low birth weight overall, it showed a beneficial effect in the high parity mefloquine group where the LBW babies were heavier than in the high parity placebo.

**Delivery Outcome**

***Congenital malformations***

Congenital malformations are defined as structural abnormalities found at birth or during the first week of life (Scrimgeour and Cockburn, 1979). The causes or factors responsible for these malformations may vary from one part of the world to the other and may be genetic or environmental. The environmental factors include race, maternal diet, vitamin intake, exposure to teratogenic drugs or environmental chemicals (e.g. pesticides), ionizing radiation, infections, metabolic imbalance (Iroha, 2001). Mefloquine, like other drugs has a potential for teratogenic effects in pregnancy. It is important to balance the risk of acquiring malaria during pregnancy and the risk of harm to the foetus and mother from the drug. Mefloquine has shown teratogenic effects in animal studies at 5-10 times the recommended dosage for humans (Phillips-Howard, 1996). However, studies conducted in Nigeria, Thailand and Malawi (Fleming, 1990; Nosten *et al.,* 1994 and Steketee *et al.,* 1996a) did not reveal any increase in the risk of teratogenicity when mefloquine was used for chemoprophylaxis in pregnancy. Smoak *et al*. (1997) did not find any major congenital malformations in US Army Servicewomen who were inadvertently exposed to mefloquine chemoprophylaxis before becoming aware of their pregnancy and Post marketing surveillance of prophylactic use of mefloquine in pregnancy also failed to demonstrate a teratogenic effect (Vanhauwere *et al.,* 1998).

The incidence and types of congenital malformations observed in this study was similar in both study and placebo groups. These findings of 16.7 per 1000 births for mefloquine and 17.4 per 1000 births for placebo were similar to findings in Lagos University

Teaching Hospital (Iroha *et al.*, 2001) where a prevalence rate of 15,8per 1000 total births was recorded, but higher than 5.5 per 1000 total births in Aminu Kano Teaching Hospital, Kano. The high incidence in this study may be as a result of data for only 60% of women enrolled in the study are represented. Congenital malformations have been shown to account for 40% of all paediatric surgery cases in ABUTH, Malumfashi (Ameh and Chirdan, 2001) and 90% of all emergency neonatal surgery in ABUTH, Zaria (Ameh *et al.*,2001).

The results of this study, provides additional data that mefloquine at prophylactic doses of 250mg weekly was not associated with the embryotoxic and teratogenic effects that have been reported in animal studies with high doses of mefloquine (Schlagenhauf, 1999).

**Haematocrit level**

Anaemia in pregnancy is a common occurrence in Nigeria and its incidence ranges from 30-60% (Sowunmi, 2003). WHO defines anaemia in pregnancy as a haematocrit value of 33% (11.0 g/dl or less). A study by Lawson, (1967) in Nigeria showed that significant harm to the foetus and mother does not occur until haemoglobin value was below 10g/dl or a haematocrit of less than 30%. As a result of this many hospital in Nigeria use lower level haemoglobin of 10g/dl or 30%, as indicating anaemia in pregnancy. The commonest causes of anaemia in pregnancy include iron and folic acid deficiency, malaria (Ogunbode and Oluboyede, 1986), hookworm infestation and HIV infection (Shulman, 1999, Hommerich *et al.*, 2007).

In this study using the WHO standard of anaemia in pregnancy, 35% of the pregnant women enrolled had anaemia at enrolment and this was found to be lower than 54% found in Ghana (Mockenhaupt *et al.,* 2000), 57% in Malawi (Rogerson *et al,* 2000) and 69% in Kenya (Oluma *et al.,* 2007). Based on haematocrit level of 30% which is considered as anaemia in pregnancy in Nigeria, only 11% of the pregnant women were anaemic at enrolment. Chemoprophylaxis increased the mean PCV in the mefloquine group. This improvement in PCV was evident as from the second month after commencement of the study and may be the most visible sign of improved protection of pregnant women against malaria by mefloquine. When grouped according to parity, the improvement in haematological parameters was only significant in the high parity mefloquine group. The low parity mefloquine group showed no significant difference in haematocrit level although there was improvement in PCV. The lack of benefit in haematological terms in this group of women may be due to the importance of other non malaria causes of anaemia such as hookworm infestation and HIV infection. Hommerich *et al.,* (2007), had similar findings in rural southern Ghana.

**Placental weight and pathology**

Placental malaria infection is recognized as a common complication of malaria in pregnancy in areas of stable transmission. A strong association between placental malaria and low birth weight has been demonstrated (Matteelli *et al.,* 1997; Steketee *et al*., 1996a), although other factors such as anaemia (Brabin *et al*., 1990) also contribute to the production of small babies. The diagnosis of placental malaria is based on the identification of parasites or malarial pigment. Parasitized red blood cells with predominantly trophozoites are found in the intervillous spaces. Malarial pigments are

found in several sites such as macrophages, trophoblasts and hoffbauer cells (Matteelli *et al.*, 1997). Clearance of pigments is within months and pigments identified in term placentas may arise from infections acquired in the second half of pregnancy. In about 50% of the placentas parasites of both groups that had pigments and parasites were also present indicating an active infection as reported by Matteelli *et al*. (1997). Fibrin deposits are indicating active and resolved infections and it is also part of the aging process of the placenta. High incidence of mild-moderate fibrin deposition seen in this study could be as a result of both active and resolved infection.

There was no difference between the placebo and mefloquine group as seen from the histopathological findings. This did not agree with the outcome of the study where the high parity mefloquine group had significantly heaver babies than placebo, and also had significantly higher haematocrit levels, during the second and third months of chemoprophylaxis indicating an improvement in protection against malaria. This discrepancy may be as a result of the small placenta sample size and the conclusion therefore as far placenta findings are concerned is inconclusive.

**Safety and tolerability of mefloquine as a chemoprophylactic agent in pregnant women**

Reports of serious neuropsychiatric side-effects associated with mefloquine prophylaxis cause some concern over prophylactic use of the drug (Bjorkman, 1989; Weinke *et al.*, 1991). Studies have shown that there was no significant difference in the incidence of serious side-effects between mefloquine and chloroquine users (Steffen *et al.*, 1993; Steketee *et al.*, 1996a). The most frequent reported adverse effects of mefloquine, which

includes dizziness, nausea, vomiting, diarrhoea, rash and pruritus were also found to be similar or lower than those observed with other antimalarials (Lobel *et al*., 1993; Croft *et al.,* 1997). It was concluded that where there is no history of seizures, psychosis or hypersensitivity to mefloquine, the drug can be used safely as a malarial chemoprophylactic agent. Overall, Mefloquine prophylaxis was well tolerated by the women in this study. No serious drug-related side-effects were reported except for one woman who withdrew from the study as a result of profuse vomiting on the day of enrolment after taking 750mg of mefloquine. Whether this vomiting was due to mefloquine, could not be confirmed. The incidence of nausea and vomiting was found to be significantly higher in the mefloquine group during the first month of enrolment in the study. A significant incidence of dizziness was reported only in the placebo group at weeks 9 and 16 of the study. Nosten *et al.* (1994), found no difference in reported side-effects between mefloquine and placebo in pregnant women except for dizziness during the first week after taking a loading dose of 500mg mefloquine. Although the women in this study took a larger loading dose of 750mg, no increase in the incidence of dizziness was observed. Long-term prophylaxis with mefloquine has not been associated with an increase in the frequency of adverse effects (Pennie *et al*., 1993; Schlagenhauf, 1999) and the frequency of these adverse effects declined with increasing duration of prophylaxis (Lobel *et al.,* 1992). This was also demonstrated in this study with the incidence of adverse effects decreasing as the number of weeks in the study increased.

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 CONCLUSION

Mefloquine chemoprophylaxis was well tolerated by pregnant women and they were protected from clinical malaria. The adverse effects experienced were self limiting. There was a significant increase in birthweight in the high parity mefloquine group and the mefloquine group also had an improvement in their haematocrit levels. In the low parity group although changes did not achieve significance, there was a general increase in all parameters studied. Placental studies showed evidence of malaria infection, but the sample size studied was small and may not be representative of the study population. From the study, mefloquine was effective for antimalarial prophylaxis in the second half of pregnancy and can be used as an alternative to SP for protection against malaria in pregnancy.

## 6.2 RECOMMENDATION

1. A study comparing mefloquine prophylaxis to IPT using sulphadoxine-pyrimethamine should be carried out to see which of them would be more beneficial.
2. A larger number of placental tissues should be used to ascertain the effect of prophylaxis with mefloquine on the placenta.
3. Distribution of insecticide-treated bed nets need to be implemented on a large scale so as to reduce exposure of pregnant women to mosquitoes and also to reduce transmission of malaria.

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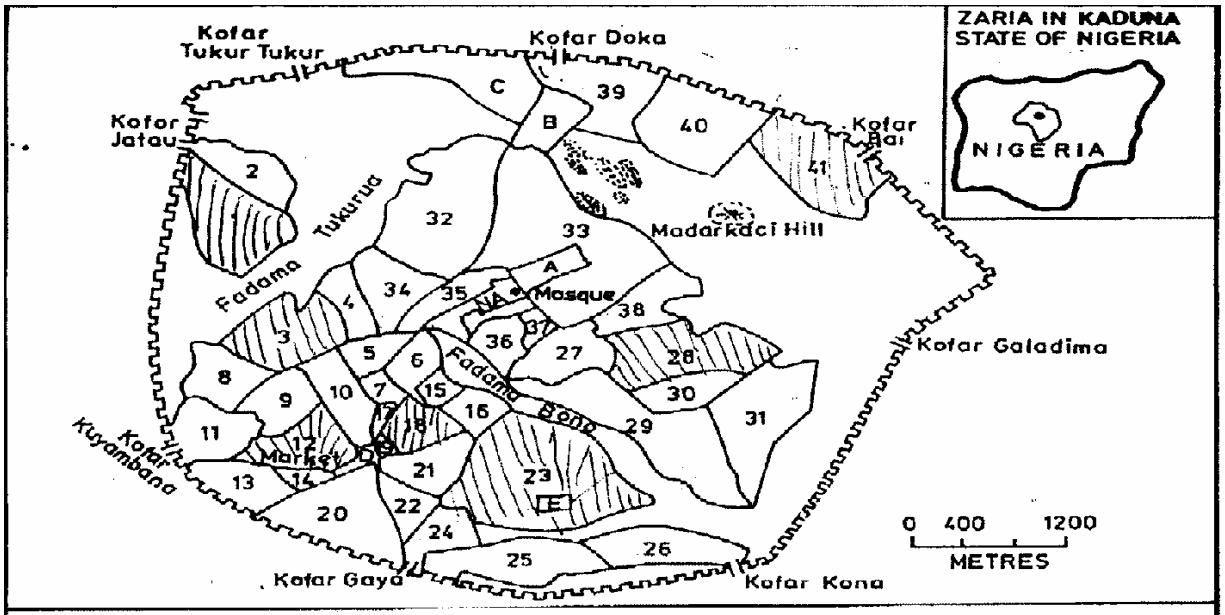
## APPENDICES

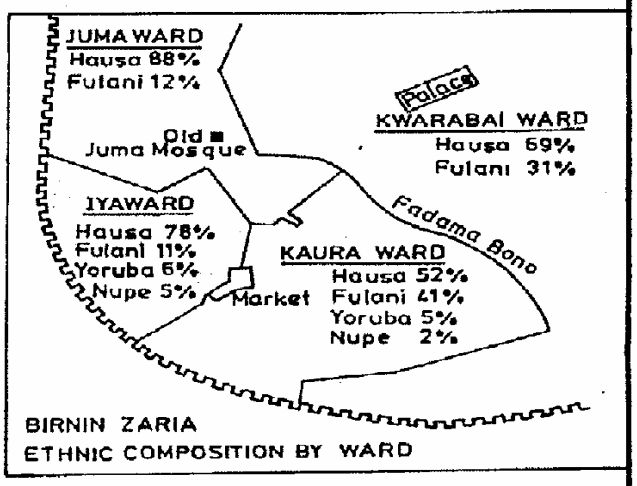
## APPENDIX I

**Map of Zaria City Local Government Area, Nigeria, showing wards chosen for the study**




UNGUWOYI (WARDS) OF ZARIA

1 Fatika
2 Sarkin Zaria
3 Juma
4 Rubu
5 Kuba or Durumin Magarke
6 Salaman Duna
7 Kanfage or Sarkin Makera
8 Barau or Kwasau
9 Alfadorai
10 Mallam Sule or Sada
11 Kofar Kuyambana
12 Limanchi or Liman Kona
13 Kusfa or Jinjiri
14 Safaiman or Chediya Biyu
15 Sirdi
16 Rimin Kambari
17 Babban Gwani
18 Nufawa
19 Melle or Mai Riga or Iya
20 Limanchin Kaura or Durumin Jamo
21 Karifi (Karfe)
22 Kurfa
23 Kaura
24 Oaz or Magaji Zakara
25 Bishar
26 Kofar Kona
27 Liman Gabdo
28 Magajiya or Madaiki
29 Magajin Aska
30 Umaru
31 Lalle
32 Alkali
33 Kwarbai or Limanin Bai or Mai Gamo
34 Dan Madami
35 Kofar Fada
36 Albarkawa
37 Madaka
38 Katuka
39 Kofar Doka
40 Jacili
41 Ban Zazzau




A= Emir's Compound
B= N.A. Prison
C= Provincial Secondary School
D= Market
E= Mallam Musa's Compound

Ward Nos. 1 to 10 Zone A
" " 11 to 20 Zone B
" " 21 to 30 Zone C
" " 31 to 41 Zone D

## APPENDIX II

## CONSENT FORM FOR PARTICIPATION IN MALARIA PREGNANCY STUDY

**SHIRIN YIN MAGANIN ZAZZABIN CIWON SAURO: HUKUMAR LAFIYA TA DUNIYA ZUWA GA DUKKAN MAZAJE DA YAN UWA DANGI**

Muna son mu sanarda kai cewa matarka……. to samu shiga cikin wadanda ake zaba cikin mata masu ciki wadanda zamu daukin nauyinsu. sabode haka zamu bata magungunan zazzabin ciwon sauro da na rigakafi kyauta. har ila yau zamu rika zuwa gidanka sau daya a cikin mako guda don kula da lafiyarta. haka kuma zamu bata wasu abubuwan da take bukata wajen haihuwa

Don haka ya zama nauyi ne kenan akan ka kabarta ta haifu anan asibitin Babban Dodo ko na Kofan Gayan don haihuwa lafiya da kuma kula da lafiyar jaririn.

DR. J.N. OKOYEH
A madadin bukumar lafiya ta Duniya, Shiyyan Zaria.

---

**BADA IZNI**

Ni……na karanta bayanin nan kuma na yarda in kyale matata …….. ta haifu a asibitin da aka ambata. kuma in ba haka ba, to na yarda in biya kudin dukkan abubuwan da aka bata.

Suna……
Ranar……..
Addreshi……
……..

## APPENDIX III

**NIGERIA**
**CCCD PROJECT**
**COMBATTING CHILDHOOD COMMUNICABLE DISEASES**

P.O. Box 1766
Kaduna, Kaduna State

September 26, 1989

Dr. L. Lege-Oguntoye
Department of Pharmacology
Ahmadu Bello University
Zaria, Kaduna State

Dear Dr. Lege-Oguntoye,

This letter is written to confirm that your research proposal entitled "Effect of Malaria Chemoprophylaxis on the Outcome of Pregnancy in Zaria, northern Nigeria" was recently approved by the Combatting Childhood Communicable Diseases Research Review Committee at its ninth meeting in Lagos on September 5, 1989.

As you are aware, the Committee is comprised of representatives of the Federal Ministry of Health, University faculty and community members. The Committee has approved your proposal from both scientific and ethical perspectives.

We appreciate your interest in conducting this important study and look forward to assisting you in the future.

Please contact me if you require any further information.

Sincerely yours,

J. Weisfeld.

Jason Weisfeld MD MPH
Secretary

# APPENDIX IV

WORLD HEALTH ORGANIZATION ORGANISATION MONDIALE DE LA SANTE

Téléphone Central/Exchange: 791.21.11
Direct: 7913737

In reply please refer to: M24/181/59
Prière de rappeler la référence: ID No. 900627

Professor L. Lege-Oguntoye
Dept of Pharmacology &
Clinical Pharmacy
Faculty of Pharmaceutical Sciences
Ahmadu Bello University
Zaria
Nigeria

7th June 1991

Project title: "The effect of malaria chemoprophylaxis on the outcome of pregnancy in Zaria, Northern Nigeria"

Dear Professor Lege-Oguntoye,

We have finally received clearance for you to begin your study referenced above using mefloquine for prophylaxis in pregnant women. Although the results of the study funded by CHEMAL have not yet been fully analyzed, Dr Davidson has written a memo based on discussions in CHEMAL, stating that there is no evidence suggesting that the drug should not be used in the last trimesters of pregnancy. A copy is attached for your information.

I hope to be able to send you the Technical Services Agreement for your signature in the near future, as soon as all the clearances have been completed.

Yours sincerely,

Dr J. Cattani
Secretary, Steering Committee
on Applied Field Research in
Malaria

cc: RD AFRO, attn: Dr D. Barakamfitiye, PM3/AFRO - please forward
National Institute for Medical Research, Yaba
Dr R.L. Kaiser, CDC, Atlanta
AFRO Liaison Office, WHO/HQ Room E.159

## APPENDIX V

**FORM 1.1**

**WHO/TDR PROJECT ON MALARIA IN PREGNANCY**
**AHMADU BELLO UNIVERSITY AND ZARIA L. G. ZARIA**

**Enrolment Form**

Date of registration:…….. Maternity Centre:……...

Name:…… S/N:……

Age:…….. Parity:…….

Husband's Name:……...

Address:…….

Participation in demographic survey: YES/NO

Ethnic group:……

Occupation:……..

Education: a. illiterate
b. primary
c. secondary
d. post-secondary

Does your husband own property? YES/NO

If yes, specify: a. land only
b. house only
c. car only
d. land and house
e. land and house and car

Do you smoke? YES/NO

If yes, how many sticks do you smoke per day?.....

Are you smoking during this pregnancy? YES/NO

If YES, how many bottles of alcoholic drink do you take per day?.....

Are you taking alcohol regularly during this pregnancy? YES/NO

Do you take drugs regularly? YES/NO

If yes, specify:……

Is there any drug which you do not usually tolerate? YES/NO

If YES, specify:…….

No. of previous pregnancies:…….

## FORM 1.2

How many previous pregnancies resulted in: a. live birth:……

b abortion:…….

c. still birth:……

d. neonatal death:……..

Did you have complications during previous pregnancies? YES/NO

If YES, specify:
a. fever
b. p.v. bleeding
c. body swelling
d. profuse vomiting
e. high blood pressure
f. other

Did you have complications during previous deliveries? YES/NO

If YES, specify: a. profuse bleeding:……

b. (pre-) eclampsia:……

c. obstructed labour:……..

d. other:…….

**CURRENT PREGNANCY**

LMP:……..

Did you have problems during this pregnancy? YES/NO

If YES, specify: a. fever
b. p. v. bleeding
c. body swelling
d. profuse vomiting
e. other

Did you have fever in the previous two weeks? YES/NO

Did you take any medication for malaria in the past 4 weeks? YES/NO

**CLINICAL EXAMINATION**

a. single foetus Saker-Solomon urine test……...

b. twins

Result of Hb electrophoresis:…….

Serum for malaria antibodies:……

**NUTRITIONAL STATUS**

Mid upper arm circumference (cm):…….

Triceps skinfold (cm):……

Pallor:…….

Hair of skin changes:…….

Other significant clinical findings:……

……..

CONSENT OBTAINED: YES/NO

CONSENT OF HUSBAND: YES/NO

**FORM 2.1**

**WHO/TDR PROJECT ON MALARIA IN PREGNANCY**
**AHMADU BELLO UNIVERSITY AND ZARIA L.G. ZARIA**

**ANC/ MATERNITY CENTRE:**

**NAME:** **S/N:** **AGE:** **PARITY:**

| | WEEKS AND DAYS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| WEEKLY | DAY 0 | 1 ( ) | 2 ( ) | 3 ( ) | 4 ( ) 1/12 | 5 ( ) | 6 ( ) | 7 ( ) | 8 ( ) 2/12 | 9 ( ) |
| MPs | | | | | | | | | | |
| No. MQ Tablets | | | | | | | | | | |
| No. P 500 Tablets | | | | | | | | | | |
| Temperature ($^{o}$C) | | | | | | | | | | |
| Course of Pregnancy) ) | | | | | | | | | | |
| Drug Toxicity ) FORM 2.3 | | | | | | | | | | |
| MONTHLY | | | | | | | | | | |
| Weeks of Gestation | | | | | | | | | | |
| Foetal HS | | | | | | | | | | |
| Breasts | | | | | | | | | | |
| Body Weight (kg) | | | | | | | | | | |
| Body Height (cm) | | | | | | | | | | |
| Pallor Y/N | | | | | | | | | | |
| Oedema Y/N | | | | | | | | | | |
| PCV (%) | | | | | | | | | | |
| BP (mmHg) | | | | | | | | | | |
| Urine Test | | | | | | | | | | |
| Drug Toxicity Tests | | | | | | | | | | |
| Fe + Fa | | | | | | | | | | |

**FORM 2.2**

| | WEEKS AND DAYS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKLY | 10 ( ) | 11 ( ) | 12 ( ) 3/12 | 13 ( ) | 14 ( ) | 15 ( ) | 16 ( ) 4/12 | 17 ( ) | 18 ( ) | 19 ( ) | 20 ( ) 5/12 |
| MPs | | | | | | | | | | | |
| No. MQ Tablets | | | | | | | | | | | |
| No. P 500 Tablets | | | | | | | | | | | |
| Temperature ($^{o}$C) | | | | | | | | | | | |
| Course of Pregnancy) ) FORM 2.3 | | | | | | | | | | | |
| Hx Toxicity ) | | | | | | | | | | | |
| MONTHLY | | | | | | | | | | | |
| Weeks of Gestation | | | | | | | | | | | |
| Foetal HS | | | | | | | | | | | |
| Breasts | | | | | | | | | | | |
| Body Weight (kg) | | | | | | | | | | | |
| Body Height (cm) | | | | | | | | | | | |
| Pallor Y/N | | | | | | | | | | | |
| Oedema Y/N | | | | | | | | | | | |
| PCV (%) | | | | | | | | | | | |
| BP (mmHg) | | | | | | | | | | | |
| Urine Test | | | | | | | | | | | |
| Drug Toxicity Tests | | | | | | | | | | | |
| Fe + Fa | | | | | | | | | | | |

**FORM 2.3**

**S/N:**

| | WEEKS AND DAYS | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ( ) | 1 ( ) | 2 ( ) | 3 ( ) | 4 ( ) | 5 ( ) | 6 ( ) | 7 ( ) | 8 ( ) | 9( ) | 10 ( ) | 11( ) | 12 ( ) | 13 ( ) |
| WEEKLY Course of Pregnancy | | | | | | | | | | | | | | |
| (a) Fever | | | | | | | | | | | | | | |
| (b) p.v. bleeding | | | | | | | | | | | | | | |
| (c) Body swelling | | | | | | | | | | | | | | |
| (d) Hyperemetis | | | | | | | | | | | | | | |
| (e) Uterine contr. | | | | | | | | | | | | | | |
| (f) Other, specify | | | | | | | | | | | | | | |
| Outside medications | | | | | | | | | | | | | | |
| Regular Fe & Fa | | | | | | | | | | | | | | |
| DRUG TOXICITY Nausea | | | | | | | | | | | | | | |
| Vomiting | | | | | | | | | | | | | | |
| Diarrhoea | | | | | | | | | | | | | | |
| Weight loss | | | | | | | | | | | | | | |
| Hair loss | | | | | | | | | | | | | | |
| Pruritas | | | | | | | | | | | | | | |
| Dizziness | | | | | | | | | | | | | | |
| Other, specify | | | | | | | | | | | | | | |
| MONTHLY WBC diff. | | | | | | | | | | | | | | |

**FORM 2.4**

**S/N:**

| | WEEKS AND DATES | | | | | |
|---|---|---|---|---|---|---|
| | 14 ( ) | 15 ( ) | 16 ( ) | 17 ( ) | 18 ( ) | 19 ( ) |
| WEEKLY<br>Course of Pregnancy | | | | | | |
| (a) Fever | | | | | | |
| (b) p.v. bleeding | | | | | | |
| (c) Body swelling | | | | | | |
| (d) Hyperemetis | | | | | | |
| (e) Uterine contr. | | | | | | |
| (f) Other, specify | | | | | | |
| Outside medications | | | | | | |
| Regular Fe & Fa | | | | | | |
| DRUG TOXICITY<br>Nausea | | | | | | |
| Vomiting | | | | | | |
| Diarrhoea | | | | | | |
| Weight loss | | | | | | |
| Hair loss | | | | | | |
| Pruritas | | | | | | |
| Dizziness | | | | | | |
| Other, specify | | | | | | |
| MONTHLY<br>WBC diff. | | | | | | |

**FORM 3.1**

**WHO/TDR PROJECT ON MALARIA IN PREGNANCY**
**AHMADU BELLO UNIVERSITY AND ZARIA L.G. ZARIA**

Name:…….

Maternity Centre:…….. S/N…….

Midwife i/c:……

Home Delivery:……. TBA i/c:…….

Age:…… Parity:…….

Date & Time of Admission:……...

ANC attendance:…… EDD……...

Duration of Labour (hours):……...

Drugs taken (if any):……..

ACTIVITIES DURING LABOUR

| Body weight (kg) | MPs | Thin film | Temp. ($^{o}$C) | BP (mmHg) | PR (per min) | PCV (%) |
|---|---|---|---|---|---|---|
| | | | | | | |

| Urine Test | Fundal Height | Fetal HS | Position |
|---|---|---|---|
| Protein:<br>Sugar: | | | |

Complication during labour: Yes/No

If Yes; specify:……...

ACTIVITIES IMMEDIATELY AFTER DELIVERY

Date & Time of Delivery:……..

Midwife i/c:……... Mode of delivery……

Complication at Delivery: Yes/No

If Yes; specify:……...

Briefly describe treatment:……

……....

Baby alive? Yes/No

**FORM 3.2**

| Thick & Thin Placental Blood smear | Thick & Thin Cord blood Smear | Cord blood (PCV) (%) | Weight of Plancenta (g) | Placenta Tissue for pathology |
|---|---|---|---|---|
| MPs:…… | MPs:…… | | | |

| BP (mmHg) | PR (per min) | Temp. ($^{o}$C) | Uterine size | Estimate Blood loss (ml) |
|---|---|---|---|---|

PLACENTA HISTOPATHOLOGY:……

ACTIVITIES AT DISCHARGE

Date & Time of discharge:……. Midwife i/c:……

| Thick & Thin Blood films | Temp. ($^{o}$C) | BP (mmHg) | PR (per min) | Body Weight (kg) | PCV (%) |
|---|---|---|---|---|---|

ACTIVITIES 6 WEEKS POST PARTUM

Date:…… Midwife i/c…….

Any complaints:…….

Drugs taken (if any):……..

Weight (kg):……...

Urine Test:…… Protein:……

Sugar:……..

Pallor:…….

BP (mmHg):……..... PR:…….per min

Temp. ($^{o}$C):…….

MPs:……...

PCV:……..

Uterine size:……..

Notes:……

……..

**FORM 3.3**

## INFANT RECORD

Names of mothers:……. S/N:……

Midwife i/c:……

### AT BIRTH

Date and Time of Birth:…….

**Apgar score:**

| | 1 min | 5 min | 10 min |
|---|---|---|---|
| Cardiovascular | ……... | ……. | …… |
| Respiratory….. | ……... | ……. | …… |
| Colour…….. | ……... | ……. | …… |
| Response…….. | ……... | ……. | …… |
| Total:…… | ……... | ……. | …… |

If delivered by TBA: (a) Cried immediately: Yes/No

Before 10 min

After 10 min

SEX: Male/Female/Uncertain

Birth Weight:……..g Length:……cm

OFC:……cm MAC:……..cm

EXAMINATION

Colour: (a) Pink

(b) Pallor

(c) Cyanosis

(d) Jaundice

Congenital anomalies (list):……...

Any injuries? (list):…….....

……

Respiration rate/min:…….

Cardiovascular rate/min:……...

**FORM 3.4**

Abdomen: Liver……cm spleen:……cm

No. of umbilical vessels……..

CNS: Moro…… Grasp……. Sucking……

**Gestational assessment:**

Total score…….. Gestation……weeks

Cord blood

MPs…… IFAT…….

Whole blood for antibodies……..

Notes:……

……

……

**COMMUNITY HEALTH WORKER'S REPORT OF HOME VISIT 2 WEEKS POST PARTUM**

Mother:……...…

……

……

Baby:……...…

……

……

6 WEEKS POST PARTUM

Any complaints……..

……

Feeding method:…….

OFC:……. MAC:……cm

Temp:…… Weight:…….g

RS:…….

CVS:……..

Abdomen:……..

CNS:……..

Other:……

…….

HEEL PRICK SMEAR

MPs:……. PCV:……

## APPENDIX VI

## History of previous pregnancies

| | Study Group | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mefloquine | | | | Placebo | | | |
| | No. of live Births | No. of Abortions | N. of Still Births | No. of Neonatal Deaths | No. of live Births | No. of Abortions | N. of Still Births | No. of Neonatal Deaths |
| | Count | Count | Count | Count | Count | Count | Count | Count |
| 0 | 154 | 418 | 465 | 453 | 132 | 357 | 422 | 412 |
| 1 | 75 | 60 | 29 | 31 | 88 | 76 | 37 | 39 |
| 2 | 81 | 12 | 2 | 11 | 57 | 15 | 2 | 8 |
| 3 | 51 | 5 | | 1 | 44 | 11 | 1 | 3 |
| 4 | 43 | 1 | | | 31 | 2 | | |
| 5 | 26 | | | | 46 | | | |
| 6 | 27 | | | | 25 | 1 | | |
| 7 | 18 | | | | 15 | | | |
| 8 | 12 | | | | 11 | | | |
| 9 | 6 | | | | 6 | | | |
| 10 | 2 | | | | 6 | | | |
| 11 | 1 | | | | 1 | | | |

## APPENDIX VII

**Number of women who either attended antenatal clinic or were visited at home for 23 weeks**

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 477(100) | 508(100) |
| 1 | 383(87.0) | 393(86.6) |
| 2 | 373(85.4) | 401(90.1)* |
| 3 | 378(86.7) | 398(89.6) |
| 4 | 391(91.6) | 404(92.2) |
| 5 | 381(90.7) | 378(90.2) |
| 6 | 371(90.5) | 376(91.5) |
| 7 | 375(92.1) | 359(88.2) |
| 8 | 356(88.8) | 372(91.9) |
| 9 | 361(91.4) | 353(89.6) |
| 10 | 349(89.3) | 349(89.5) |
| 11 | 347(89.2) | 333(87.2) |
| 12 | 338(88.5) | 342(91.0) |
| 13 | 315(85.4) | 323(89.0) |
| 14 | 328(90.4) | 305(88.7) |
| 15 | 294(86.2) | 292(89.8) |
| 16 | 302(91.8) | 278(92.7) |
| 17 | 280(92.7) | 231(86.5)* |
| 18 | 240(90.9) | 219(88.7) |
| 19 | 193(85.0) | 163(80.3) |
| 20 | 176(89.3) | 155(92.3) |
| 21 | 129(89.6) | 109(90.1) |
| 22 | 90(82.6) | 70(85.4) |
| 23 | 70 (88.6) | 45(81.8) |

***p<0.05**

- **Number in parenthesis are percentages**

## APPENDIX VIII

**Incidence of malaria infection in pregnancy women at enrolment and break through parasitemia during chemoprophylaxis with 250mg mefloquine weekly until delivery**

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 33(6.9)*** | 6(1.2) |
| 1 | 3(1.0) | 0(0) |
| 2 | 1(0.3) | 1(0.3) |
| 3 | 1(0.3) | 0(0) |
| 4 | 9(2.4)* | 2(0.5) |
| 5 | 0(0) | 2(0.6) |
| 6 | 2(0.7) | 3(0.9) |
| 7 | 2(0.6) | 1(0.4) |
| 8 | 1(0.3) | 1(0.3) |
| 9 | 8(2.7)** | 0 |
| 10 | 3(1.0) | 0 |
| 11 | 4(1.5) | 2(0.8) |
| 12 | 3(1.0) | 1(0.3) |
| 13 | 2(0.8) | 1(0.4) |
| 14 | 7(2.6) | 2(0.8) |
| 15 | 1(0.4) | 1(0.4) |
| 16 | 1(0.4) | 0 |
| 17 | 2(0.9) | 0 |
| 18 | 1(0.5) | 0 |
| 19 | 0 | 1(0.8) |
| 20 | 1(0.6) | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 1(1.6) | 0 |

***p=0.03 **p=0.008 fishers exact test ***p=0.0001**

- **Numbers in parenthesis are percentages**

## APPENDIX IX

**Incidence of fever in pregnant women of the mefloquine and control group, during chemoprophylaxis**

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 83(17.5) | 65(13.2) |
| 1 | 26(6.8) | 18(4.5) |
| 2 | 15(4.0) | 10(2.5) |
| 3 | 14(3.7) | 12(3.0) |
| 4 | 20(5.1) | 10(2.5)***** |
| 5 | 8(2.1) | 3(0.8) |
| 6 | 10(2.7) | 3(0.8)***** |
| 7 | 8(2.1) | 0****** |
| 8 | 12(3.4) | 8(2.2) |
| 9 | 7(1.9) | 2(0.6) |
| 10 | 4(1.1) | 2(0.6) |
| 11 | 5(1.4) | 2(0.6) |
| 12 | 4(1.2) | 3(0.9) |
| 13 | 4(1.3) | 6(1.8) |
| 14 | 3(0.9) | 3(1.0) |
| 15 | 2(0.7) | 3(1.0) |
| 16 | 7(2.3) | 3(1.1) |
| 17 | 2(0.7) | 1(0.4) |
| 18 | 1(0.4) | 1(0.5) |
| 19 | 1(0.5) | 2(1.2) |
| 20 | 3(1.7) | 0 |
| 21 | 2(1.5) | 0 |
| 22 | 1(1.1) | 0 |
| 23 | 2(2.8) | 0 |

***p=0.05, **p=0.01**

- **Numbers in parenthesis are percentages**

## APPENDIX X

### Incidence of nausea in pregnant women on weekly mefloquine Chemoprophylaxis

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 94(20.7) | 100(21.0) |
| 1 | 15(3.9) | 24(6.0) |
| 2 | 12(3.2) | 15(3.8) |
| 3 | 9(2.4) | 13(3.3) |
| 4 | 11(2.8) | 23(5.7) ***** |
| 5 | 8(2.1) | 10(2.6) |
| 6 | 2(0.5) | 5(1.3) |
| 7 | 3(0.8) | 3(0.8) |
| 8 | 7(2.0) | 6(1.6) |
| 9 | 1(0.3) | 4(1.1) |
| 10 | 1(0.3) | 2(0.6) |
| 11 | 2(0.6) | 3(0.9) |
| 12 | 3(0.9) | 5(1.5) |
| 13 | 1(0.3) | 1(0.3) |
| 14 | 3(0.9) | 1(0.3) |
| 15 | 2(0.7) | 2(0.7) |
| 16 | 1(0.3) | 2(0.7) |
| 17 | 1(0.4) | 4(1.7) |
| 18 | 0 | 2(0.9) |
| 19 | 0 | 0 |
| 20 | 2(1.1) | 2(1.3) |
| 21 | 0 | 0 |
| 22 | 0 | 1(2.1) |
| 23 | 1(1.4) | 1(2.1) |

***p=0.04**

- **Numbers in parenthesis are percentages**

## APPENDIX XI

**Incidence of vomiting among pregnant women taking mefloquine 250mg weekly and placebo**

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 50(12.3) | 45(10.4) |
| 1 | 8(2.1) | 2(5.6)* |
| 2 | 7(1.9) | 18(4.7)** |
| 3 | 5(1.3) | 11(2.8) |
| 4 | 11(2.9) | 23(6.1)*** |
| 5 | 5(1.3) | 4(1.1) |
| 6 | 1(0.3) | 1(0.3) |
| 7 | 3(0.8) | 1(0.3) |
| 8 | 2(0.6) | 2(0.5) |
| 9 | 2(0.6) | 3(0.9) |
| 10 | 1(0.3) | 4(1.2) |
| 11 | 1(0.3) | 2(0.6) |
| 12 | 1(0.3) | 4(1.1) |
| 13 | 0 | 1(0.3) |
| 14 | 1(0.3) | 0 |
| 15 | 1(0.3) | 0 |
| 16 | 0 | 0 |
| 17 | 1(0.4) | 2(0.9) |
| 18 | 0 | 1(0.5) |
| 29 | 0 | 1(0.6) |
| 20 | 1(0.6) | 1(0.7) |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |

***p=0.02 **p=0.04 ***p=0.04**

- **Numbers in parenthesis are percentages**

## APPENDIX XII

**Incidence of diarrhoea in pregnant women taking mefloquine 250mg weekly**

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 9(2.0) | 9(1.9) |
| 1 | 3(0.8) | 3(0.8) |
| 2 | 3(0.8) | 3(0.8) |
| 3 | 2(0.5) | 2(0.5) |
| 4 | 2(0.5) | 5(1.3) |
| 5 | 4(1.1) | 1(0.3) |
| 6 | 1(0.3) | 1(0.3) |
| 7 | 2(0.5) | 1(0.3) |
| 8 | 2(0.5) | 1(0.3) |
| 9 | 0 | 1(0.3) |
| 10 | 1(0.3) | 1(0.3) |
| 11 | 0 | 1(0.3) |
| 12 | 0 | 3(0.9) |
| 13 | 0 | 0 |
| 14 | 0 | 1(0.3) |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 1(0.4) | 0 |
| 18 | 0 | 3(1.4) |
| 19 | 1(0.5) | 2(1.3) |
| 20 | 1(0.6) | 2(1.3) |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |

- **Numbers in parenthesis are percentages**

## APPENDIX XIII

**Incidence of dizziness in pregnant women taking mefloquine (250mg) weekly**

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 75(19.7) | 63(15.2) |
| 1 | 17(4.5) | 17(4.7) |
| 2 | 6(1.6) | 8(2.0) |
| 3 | 8(2.2) | 9(2.3) |
| 4 | 7(1.8) | 12(3.1) |
| 5 | 7(1.9) | 5(1.3) |
| 6 | 5(1.4) | 2(0.5) |
| 7 | 6(1.6) | 1(0.3) |
| 8 | 3(0.8) | 3(0.9) |
| 9 | 4(1.1) | 0***** |
| 10 | 1(0.3) | 1(0.3) |
| 11 | 3(0.9) | 2(0.6) |
| 12 | 2(0.6) | 2(0.6) |
| 13 | 1(0.3) | 0 |
| 14 | 3(1.0) | 1(0.3) |
| 15 | 2(0.6) | 2(0.7) |
| 16 | 5(1.7) | 0****** |
| 17 | 1(0.4) | 0 |
| 18 | 1(0.4) | 0 |
| 19 | 1(0.5) | 0 |
| 20 | 2(1.1) | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |

***p= 0.049 **p=0.03**

- **Numbers in parenthesis are in percentages**

## APPENDIX XIV

**Frequency of pruritus in pregnant women taking mefloquine 250mg and placebo over 23 weeks**

| Week | Placebo | Mefloquine |
|---|---|---|
| 0 | 19(4.4) | 22(4.9) |
| 1 | 8(2.1) | 5(1.3) |
| 2 | 4(1.1) | 4(1.0) |
| 3 | 3(0.8) | 6(1.8) |
| 4 | 8(2.1) | 7(1.8) |
| 5 | 5(1.3) | 4(1.1) |
| 6 | 5(1.4) | 3(0.8) |
| 7 | 3(0.8) | 2(0.6) |
| 8 | 2(0.6) | 2(0.5) |
| 9 | 1(0.3) | 3(0.8) |
| 10 | 2(0.6) | 1(0.3) |
| 11 | 0 | 2(0.6) |
| 12 | 0 | 0 |
| 13 | 0 | 0 |
| 14 | 0 | 1(0.3) |
| 15 | 2(0.7) | 1(0.4) |
| 16 | 0 | 0 |
| 17 | 0 | 1(0.5) |
| 18 | 0 | 0 |
| 19 | 0 | 0 |
| 20 | 0 | 0 |
| 21 | 0 | 0 |
| 22 | 0 | 0 |
| 23 | 0 | 0 |

- **Numbers in parenthesis are percentages**