A RANDOMISED, CONTROLLED TRIAL OF THREE DRUG REGIMES FOR THE TREATMENT OF MALARIA IN PREGNANCY IN GHANA.

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BSc. MB ChB

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A RANDOMISED, CONTROLLED TRIAL OF THREE DRUG REGIMES FOR THE TREATMENT OF MALARIA IN PREGNANCY IN GHANA.

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A thesis presented on a clinical trial of amodiaquine (AQ) and sulphadoxine-pyrimethamine (SP) used singly and in combination (AQ+SP) compared with chloroquine (CQ) for the treatment of 900 pregnant women who had falciparum malaria infection detected by a screening programme using OptiMAL® antigen dipsticks during routine antenatal clinic sessions at the St. Theresa's Hospital. Enrolment into the study began in March 2003 and ended in September 2004 but follow up of treated women continued to March 2005.

DEDICATION

Dedicated to my wife Ama and children Elorm, Edem, Eyram and Elikem.

ABSTRACT

Malaria in pregnancy is potentially fatal to both the mother and the foetus particularly in the primigravidae warranting prompt diagnosis and efficacious treatment. Amodiaquine (AQ) alone or its combination with sulphadoxine-pyrimethamine (SP) may be suitable options for treatment as the spread of *P. falciparum* resistance to CQ and SP increases while the safety of the newer antimalarials including the artemisinins remain largely untested in pregnant women. We conducted a randomised controlled trial to assess the efficacy, safety and tolerability of AQ and SP alone or their combinations compared with CQ in pregnant women.

Pregnant women of all parities attending antenatal clinic sessions at St. Theresa's Hospital in the Nkoranza district of Ghana with a gestational age of 16 weeks and above were screened for malaria antigens with OptiMAL® dipsticks. Two hundred and twenty five pregnant women with positive antigen tests and parasitaemia confirmed microscopically were enrolled into each of four arms of the study. The women were followed up on days 3, 7, 14 and 28 after the start of treatment to assess the effect of treatment on peripheral parasitaemia, haemoglobin, bilirubin, liver transaminases and white cell counts. In addition, participants' reports of adverse effects were recorded and monitored during the follow-up visits. Parasitological, haematological and neonatal outcomes were also assessed at delivery and six weeks postpartum.

The parasitological failure up to day 28 was, 29.8% (62/208), 16.4% (34/ 208), 7.5% (16/ 212) and 2.4% (5/ 210) in the CQ, SP, AQ and the AQ+SP groups respectively. The parasitological failure corrected for new infections using msp-2 genotyping, was 14.4%, 10.6%, 2.8% and 1.0% the CQ, SP, AQ and the AQ+SP groups respectively..

The mean Hb increased by 0.4g/dl (95% CI; 0.3 to 0.5 $p<0.001$) and 0.9g/dl (95% CI; 0.8 to 1.0 $p<0.001$) by day 14 and 28 respectively relative to the baseline mean Hb.

At day 3, 75% (625/869) of women reported an adverse event of which general weakness, dizziness, vomiting, itching and nausea were most frequent. Compared to women who received CQ, women in the AQ (OR=1.9, 95% CI, 1.7 - 3.2 p=0.01) and the AQ+SP (OR=3.0; 95% CI, 1.7 - 5.1 p<0.001) groups were more likely to report an adverse effect; women in the SP group were less likely to report an adverse event OR=0.3; 95% CI, 0.2 - 0.4 p<0.001).

The mean activities of aspartate and alanine aminotransferase increased relative to enrolment levels at day 14 but decreased towards baseline levels at day 28 in all the treatment groups. On the contrary the mean activity of gamma glutamyl transferase increased throughout up to day 28, but this increase was within normal range in all the treatment groups. The mean concentrations of total bilirubin and its fractions decreased both at day 14 and day 28 relative to baseline concentrations. The neutrophil counts increased after the start of treatment relative to baseline counts in all the treatment groups but overall, there was no significant difference between mean neutrophil counts at follow up days and the mean count at enrolment (p>0.05).

We concluded that AQ alone or its combination with SP although associated with minor adverse events, are efficacious and safe and, could be one of the antimalarial treatment options available to pregnant women with *P. falciparum* malaria.

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I am greatly indebted to Dr Daniel Chandramohan and Professor Brian Greenwood for the invaluable support, encouragement and supervision they gave me for the successful implementation and running of the field project and the preparation of this thesis.

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I also thank Mr Gyimah of the Mathematics Department of the University of Science and Technology, Kumasi who generated the random numbers and together with his staff labelled each individual drug pack with a number code to ensure blinding.

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I wish to thank Mr. Ampofo Twumasi, the District Chief Executive of the Nkoranza District Assembly who organised members of the assembly for me to brief them at the beginning of the project and to solicit their support in their electoral areas.

I am also greatly indebted to the following at the LSHTM: Anna Randall and Ros Ords for analysing blood spots for PCR genotyping of the parasites; and Dr Kaur Harparkash for confirming the solubility and content of the study drugs using the high pressure liquid chromatography (HPLC).

Finally, I wish to express my heartfelt gratitude the pregnant women who participated and their families for accepting the challenge to contribute to the efforts of searching for efficacious and safe antimalarials drugs for use in pregnancy.

I am grateful to the Gates Malaria Partnership, LSHTM, UK for the full sponsorship provided for the entire costs involved in tuition, field project and travel towards the successful completion of my doctoral thesis. My sincere thanks also go to all the good people at the GMP secretariat past and present who ensured that logistics and funds reached me timely throughout the period.

I also acknowledge with gratitude the support of numerous others whose names have not been mentioned here due limitations imposed by space. Thank you all.

ABBREVIATIONS

AE (s)	Adverse Event (s)
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
AOR	Adjusted Odds Ratio
AST	Aspartate Aminotransferase
CRF(s)	Case Record Form (s)
CRS	Catholic Relief Services
DSMB	Data Safety Monitoring Board
EDTA	Ethyl Diamine Tetra Acetate
GARFUND	Ghana AIDS Relief Fund
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HPLC	High Pressure Liquid Chromatography
IQR	Inter Quartile Range
ITNs	Insecticide treated mosquito net(s) or material (s)
LR test	Likelihood ratio test
LSHTM	London School of Hygiene and Tropical Medicine
MCH	Maternal and Child Health
OPD	Outpatients Department
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PI	Principal Investigator
pLDH	Plasmodium Lactate Dehydrogenase
PLWHAS	People living with HIV/AIDS
SAE(s)	Serious Adverse Event(s)
SD	Standard Deviation
SP	Sulphadoxine Pyrimethamine
TBA	Traditional Birth Attendant
WBC	White blood cell
WHO	The World Health Organisation

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CHAPTER ONE

INTRODUCTION

CHAPTER 1 INTRODUCTION

1.1 DrPH Integrating Statement

I have a medical background and have also been involved in health services management since my appointment as the director of health services of the Nkoranza district in Ghana in August 1995. I also doubled up as a resident senior medical officer of the St. Theresa's Hospital, Nkoranza. I also led a local team to successfully implement a model safe motherhood programme initiated by MaterCare International and funded by Canadian International Development Agency (CIDA).

I won a GMP studentship in 2001 to study for the Doctor of Public Health programme at the London School of Hygiene and Tropical Medicine. The first part involved passing taught courses in evidence based public health practice, statistics and epidemiology run at the LSHTM from October 2001 to May 2002.

From July – September 2002, I undertook a professional attachment (PA) with the National Malaria Control Programme (NMCP) of Ghana. The purpose of the attachment was to analyse the structure and work of the NMCP and its achievements in reducing the malaria burden in Ghana from 1992 to 2002. Specifically, it aimed to: (1) describe the operations of the NMCP; (2) analyse and characterize the public health impact of the activities of the NMCP; (3) explore the strength, weaknesses, opportunities and threats of the current structure of the NMCP in responding to the Global Malaria Control Strategy and the RBM initiative. During the PA, I (1) shadowed the NMCP manager; (2) observed malaria control programme operations at the district and regional levels; (3) interviewed key informants directly connected with the NMCP; (4) reviewed several documents on Ghana's health sector and malaria control activities. A report of the attachment was presented to the LSHTM.

In August 2002 during my PA, the NMCP organised a national forum for health

professionals and research scientists to discuss the revision of the national antimalarial drug policy. A change to amodiaquine combination with artesunate or amodiaquine with sulphadoxine-pyrimethamine as first line treatments for uncomplicated malaria was considered. But there was no local data then to support any change.

This event created the opportunity for me to develop a proposal for a randomised controlled trial of amodiaquine and sulphadoxine-pyrimethamine used singly or in combination for the treatment of malaria infection in pregnancy. This proposal was reviewed and approved by the research committee of the GMP and ethical approval was given by the Health Research Unit of the Ministry of Ghana and the London School of Hygiene and Tropical Medicine. The data generated from the research project forms the basis of this thesis.

I was the principal investigator for the project and was responsible for: -

- Designing the research protocol and standard operating procedures and responded to all queries from research review committee of the GMP and ethical committees in Ghana and in London.
- Designing study drugs formulations and packaging in collaboration with Kinapharma Ltd of Ghana, the drug manufacturers to ensure blinding.
- Setting up project office at the St. Theresa's hospital mainly for project administration and data entry and management.
- Recruiting and managing the project staff.
- Conducting the initial and continuous practical skill-training courses for the field teams and data management team for them to understand the nature of the project and acquire knowledge and skills needed for their work.
- Developing suitable record forms and database for the project.

- Participant enrolment and the supervising the entire project throughout the study period.
- Managing the project data throughout the study period.
- Wrote a statistical analysis plan which was approved by DSMB.
- Analysing the trial data and writing the thesis.

During the study period, I spent between one to two weeks in the laboratory to have hands on experience of the techniques involved in PCR genotyping at the Kintampo Health Research Centre and LSHTM, blood film microscopy and clinical chemistry analysis.

Dissemination of study results so far:

1. Three posters on "Safety and efficacy of amodiaquine and sulphadoxine-pyrimethamine SP in Ghanaian pregnant women" have been presented at the annual meetings of BSP in Chester, UK in 6 to 10th April 2004; of ASHTM in Miami, USA held from 7 to 11th November 2004 and at the 4th MIM conference in Yaoundé, Cameroon held from 13 to 18th November 2005.
2. Oral presentation of general findings at the annual expert oversight committee of the Gates Malaria Partnership (GMP) meeting in Banjul, the Gambia held from 10 to 14th May 2005.

1.2 Study rationale

Plasmodium falciparum infection in pregnancy is associated with an increased risk of maternal anaemia, abortion, stillbirth, prematurity, intra-uterine growth retardation and low birth weight. Preventive and control measures are available but the rates of maternal and foetal complications are still very high. This is because of the low uptake of malaria control programmes the wide spread and increasing *P. falciparum* resistance to the cheap and commonly available chloroquine and sulphadoxine-pyrimethamine. Apart from insecticide treated bed nets (ITNs), the success of any chemoprophylaxis, intermittent preventative

(IPT) or treatment programme depends heavily on the efficacy and safety of available antimalarial drugs. Unfortunately for pregnant women, the options for efficacious and safe antimalarials drugs are limited because they were always excluded from most drug trials for safety reasons. The introduction of new antimalarial regimes and changes in national drug policies means that pregnant women will advertently or inadvertently be exposed to these drugs whose safety profiles in pregnancy are largely unknown. Clinical trials, designed to investigate the efficacy and safety of affordable antimalarial drugs to replace CQ and SP for use in pregnancy are therefore warranted. Prior to this study, the national antimalarial drug policy for Ghana was chloroquine as first line drug both for treatment and prophylaxis in pregnant women. Discussions were ongoing to change the policy most probably to a combination regime of amodiaquine plus artesunate or amodiaquine plus SP. Evidence on the efficacy and safety of these regimes was lacking locally particularly for pregnant women. There were concerns over the safety of AQ when used in pregnancy. Whether or not, on balance, the potential benefits of these treatments outweigh the potential risks could only be determined in a prospective, randomised clinical trial.

In anticipation of the withdrawal of chloroquine as the drug of choice for malaria treatment and the introduction of combination therapy involving AQ and SP for malaria, exploration of their efficacy and safety alone or in combination therapy in pregnant women was necessary and urgent. This study was therefore designed in response to the local search for an appropriate, efficacious and safe antimalarial drug regime for use in pregnant women.

1.3 Study hypotheses

1. Amodiaquine (AQ) and sulphadoxine-pyrimethamine (SP) used singly or in combination (AQ+SP) are significantly superior to chloroquine (CQ) in achieving parasitological clearance in Ghanaian pregnant women with antenatal *Plasmodium falciparum* malaria infection.

2. Amodiaquine (AQ) and sulphadoxine-pyrimethamine (SP) used singly or in combination (AQ+SP) to treat antenatal malaria infection are as safe as chloroquine (CQ).

1.4 Organisation of the thesis

The thesis is divided into the following chapters: -

Chapter 1 Introduction to the thesis

This chapter presents the rationale and hypotheses of the study and provides a general overview of the organisation of the thesis.

Chapter 2 Literature review

This chapter presents a brief review of the global malaria situation and the epidemiology of malaria and its control in Ghana. This is followed by a review of the epidemiology of malaria in pregnancy, its pathogenesis, clinical features, diagnosis and control. A brief summary of the properties of the study drugs are also presented including known adverse drug events. Finally a brief review of how the efficacy and safety of antimalarial drugs used in pregnancy have been evaluated is presented.

Chapter 3 Objectives, patients, methods and materials

This chapter outlines the objectives and design of the study and provides a description of the study site and population. It also describes the pre-trial activities, ethical issues and the overall conduct of the trial including procedures used.

Chapter 4 Results 1: - Screening and enrolment

The first section of this chapter provides a description of the screening processes that led to randomisation of eligible pregnant women into the study. The second section presents a

description of the demographic and social characteristics of the study women, their clinical and obstetric assessments and baseline parasitological and haematological indices.

Chapter 5 Results 2: - Treatment outcomes

This chapter presents the parasitological and haematological outcomes during the initial 28 days of follow up after the start of treatment. The proportions of parasitological failure in the AQ, SP and AQ+SP groups are compared to failures in the CQ group at days 14 and 28 of follow up. Mean changes in haemoglobin over enrolment levels at days 14 and 28 are compared within and between treatment groups. The chapter also describes the relationships between baseline prognostic factors and the parasitological and haematological outcomes of treatment.

Chapter 6 Results 3: - Safety and tolerance

This chapter presents the results of the safety and tolerance observations made among the study pregnant women over the 28-day follow up period after the start of treatment. The first section describes the types and incidence of adverse effects reported by women during post treatment follow up at days 3, 7, 14 and 28 and makes comparisons between treatment groups. A comparison is also made of the incidences of complaints obtained from a control group of untreated non-parasitaemic pregnant women who were screened at the same clinic and received the same number of follow up visit with those of enrolled parasitaemic pregnant women. The second section describes the levels of liver enzymes and bilirubin at days 0, 14 and 28 and makes comparisons between the treatment groups. This section also compares the white blood cell counts at days 0, 3, 7, 14 and 28 based on a reference range of 4.0 - 11.0 x 10^9/L within treatment groups and in all treatment groups compared with CQ.

Chapter 7 Results 4: - Pregnancy outcomes

This chapter presents results on the parasitological and haematological outcomes at delivery and postpartum. These outcomes are presented initially as the estimates of the treatment effect and then as an overall effect compared to baseline levels. There is also a descriptive

analysis of birth outcomes from women in the study and compared by treatment group. Also the overall incidences of adverse pregnancy outcomes in the study group are compared to local rates for abortion, stillbirth, congenital abnormality, and mean gestation at delivery obtained from St Theresa's Hospital's records.

Chapter 8 Discussions and conclusions

This chapter presents a discussion of the main study findings and their limitations within the context of what is already known. The implications of the findings for further research and policy for the control of malaria in pregnancy generally and for Ghana are presented.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER 2 LITERATURE REVIEW

2.1 Global burden of malaria

The disease burden of malaria may be underestimated as data used in such computations are obtained mainly from the formal health system, excluding figures from the informal health sector (Sauerborn *et al.* 1991; Shepard *et al.* 1991; RBM/WHO 1999). Factors contributing to the high burden of malaria include the prevalence of *Plasmodium falciparum*, presence of *Anopheles gambiae*, poverty, failures in health systems, rapidly emerging resistance to many drugs, war and refugee situations, poor sanitation, unplanned development activities in developing countries, and climatic changes (Brabin *et al.* 2001). These factors are more prevalent in Africa south of the Sahara which probably explains why the greatest burden of malaria is found in this region of the world (Snow *et al.* 1999). About forty percent of the world's population are at risk of malaria and nine out of ten cases come from Africa south of the Sahara (WHO 2000b). Children and pregnant women are most vulnerable and more likely to suffer the fatal complications of malaria including anaemia, hypoglycaemia and cerebral damage. About one million people are killed each year by malaria, the overwhelming majority of whom are children aged 5 years or younger. Ninety percent of malaria deaths occur in rural sub-Saharan Africa (WHO 2000b). Severe malaria in children under five years of age is also known to impact negatively on the development of children through neuropsychological impairment, poor growth and limitations in school achievements. However, the contributions of these to the malaria burden remain unclear (Holding and Kitsao Wekulo 2004). One of two reviews of studies reporting neuropsychological sequelae estimated that at least 1000 children are likely to suffer serious neurological deficits following cerebral malaria attacks each year (Mung'Ala Odera *et al.* 2004); the second review reported figures of 9000 to 19,000 (Murphy and Breman 2001).

Malaria is both a cause and consequence of the poor socio-economic situation found in most of Africa south of the Sahara, which is one of the poorest regions of the world (RBM/WHO

1999). Malaria imposes suffering and poverty on individuals, households and governments of endemic countries (Bloland *et al.* 1996; RBM/WHO 1999; Breman 2001; Breman *et al.* 2001). Income levels of malaria endemic countries are about a third less than those of countries without malaria. The estimated annual direct and indirect costs of malaria in Africa are more than 2000 million US dollars per year (Gallup and Sachs 2001; Sachs and Malaney 2002). Malaria is the cause of up to fifty percent of outpatient diagnoses and up to twenty percent of admissions at an estimated cost of $1.10 per outpatient visits in Malawi and $35 per admission in Kenya (RBM/WHO 1999). Families spend an average of 25% of their annual income on treatment, as well as paying costs for prevention and suffering loss of income. The costs of treating malaria for small farmers in Kenya and Nigeria have been estimated to be as high as 5% and 13% of total household expenditure respectively (RBM/WHO 1999). The cost of treating malaria accounts for about 3% of the average household income monthly and about half of curative health care costs incurred by households in Nigeria (Onwujekwe *et al.* 2000). Infection with malaria results in loss of productivity both in the formal and non-formal sectors of the economies of endemic countries (Bloland *et al.* 1996; Onwujekwe *et al.* 2000; Breman *et al.* 2001; Gallup and Sachs 2001; Sachs and Malaney 2002). Available evidence suggests that the adverse economic impact of malaria in Africa is greater than 1% of gross domestic product (GDP). For Kenya the overall production loss is estimated to be 2–6% of GDP, figures for Nigeria are 1 – 5% (RBM/WHO 1999).

Resistance to anti-malarial drugs is a significant public health problem for many malaria control programmes because parasites have demonstrated some level of resistance to almost every antimalarial drug currently available (Bloland and Ettling 1999; D'Alessandro and Buttiens 2001). Of particular worry is the rapid spread of resistance of *P. falciparum* to chloroquine and SP throughout sub-Saharan Africa. This has epidemiological, clinical and policy implications for malaria control in Africa. An example of the magnitude of the problem of falciparum resistance in eastern Africa is shown in **Table 2.1**. Increases in the

failure rates of CQ and SP monotherapies have necessitated reviews of national antimalarial drug policies across the East African sub region (EANMAT 2003).

Table 2.1: - Clinical failure rates of chloroquine and SP in East Africa between 1998 and 2001.

Country	Population (million)	Estimated number of malaria cases per year (million)	Resistance to CQ (%)	Resistance to SP (%)
Burundi	6.5	2	50-90	13-63
Kenya	30	8.2	66-87	27-40
Rwanda	7.2	1.2	40	16-45
Tanzania	32.8	8.6	28-72	15-34
Uganda	21.1	5.3	10-80	11-60

Adapted from "PRESS DOSSIER: Changing national malaria treatment protocols in Africa". February 2002, Nairobi, Kenya.

Some studies also suggest that the spread of chloroquine resistance has led to a dramatic increase in malaria mortality in most epidemiological contexts in tropical Africa (Zucker *et al.* 1996; Trape 2001).

Antimalarial drug resistance may contribute to the spread of malaria to new areas, to the re-emergence of malaria in areas where the disease had been eradicated and to the occurrence and severity of epidemics in some parts of the world (Bloland *et al.* 1993; Barat *et al.* 1998; Bloland *et al.* 1998; Bloland *et al.* 2000).

2.2 Malaria in Ghana

2.2.1 Demographic and socioeconomic features

Source: - http://www.afro.who.int/malaria/country-profile/ghana.pdf

Ghana is situated on the West Coast of Africa, and is bordered by Burkina Faso to the North, the Gulf of Guinea (537km) to the South, Togo to the East and Côte d'Ivoire to the West. It covers an area of 238,538km^2. It has a tropical climate with a temperature range from 18°C to 40°C. There are two main seasons — wet and dry. In the north, the wet season is short and lasts from May to August while the dry season increases in intensity with each month from September to April. In the south, the wet season, lasts from April to November, and is interrupted by a short dry spell in August. The annual rainfall, which ranges from 700mm in the coastal area and the north to over 2000mm in the forest belt, delineates the country topographically into three ecological zones namely; the southern coastal plains, the rainforest middle belt and the savannah north. Ghana has a population of approximately 20 million with an annual growth rate of 2.5% according to the 2000 Census, with an annual growth rate varying from region to region (GSS/NMIMR/ORC 2004).

The budgetary allocation for the Ministry of Health was 699.4 billion cedis for 2002 (approximately US$ 93.2 million). However, government's budgeted per capita expenditure on health for the year 2002 was US$6.90, as against the Commission on Macro Economics and Health's recommended US$34 per capita per year for health. The health budget is

currently between 8% and 12% of Central Government expenditure and ranks third after education and agriculture. These resources are inadequate and remain a major constraint to health services provision. The private, non-governmental, multilateral and bilateral agencies contribute greatly to the provision of health services, especially in the rural areas (MOH 1997; MOH 2002a; MOH 2002b).

Ghana's health system is based on the Primary Health Care (PHC) approach in which functional District Health Management Teams (DHMT), as decentralized units, are responsible for the implementation and management of health policies and programmes. Each of the 110 districts in Ghana has a functional District Health Management Team (DHMT), but their capacities to efficiently plan, implement and monitor programmes vary considerably. The Ghana Health Service provides curative and preventive/promotive services at national, regional, district and sub district levels. The services are provided through a network of 172 hospitals, 1056 health centres/clinics and 361 maternity homes. These service delivery points include public, private for profit, private non-profit as well as the military, police and tertiary hospitals.

2.2.2 Epidemiological pattern of malaria in Ghana

Malaria and its outcomes in Ghana have been observed to vary with the prevailing season and the ecology of the country. Binka and others showed marked seasonal variations among children in northern Ghana, with parasite rates reaching 85% to 94% in the wet season (Binka *et al.* 1994). They found parasite rates to be highest in children aged 5-7 years, while parasite densities and rates of febrile illness were highest in those 6-11 months old and associated with low levels of haemoglobin. In southern Ghana, Afari and others showed that the crude parasite rates ranged from 19.6% to 33.5% in the dry season and from 33% to 44% in the wet season. *Plasmodium falciparum* was found to be the predominant parasite species with high prevalence rates (up to 96%) in the rainy season compared to 80% in the dry

season. The prevalence of *P. malariae* was 20.4% and that of *P. ovale* 2.7% (Afari *et al.* 1992; Afari *et al.* 1993). Mixed infection rates were found more frequently in the dry season.

In a community-based survey of 35 villages, Browne and others showed that the overall prevalence of malarial parasitaemia in subjects aged 2 years and above were 50.7% in the forest areas and very similar (49.7%) in the savannah areas of Ghana with *P. falciparum* being the most prevalent in all the ecological zones. *P. malariae* and *P. ovale* were found more commonly in the savannah and forest zones respectively. Asexual parasitaemia (of any species) was highest in the youngest age group of 2 to 9 years and then decreased with age. Mixed infections were observed in 24% and 30% of the parasitaemic subjects from the forest and savannah, respectively (Browne *et al.* 2000).

In Ghana, it is estimated that malaria, measles, childhood pneumonia, sickle cell disease and severe malnutrition are the 5 most important causes of loss of healthy lives and between them account for 34% of healthy lives lost due to all diseases. Malaria is the first cause of morbidity accounting for about 40% of all OPD attendance (Binka *et al.* 1994). It is also the leading cause of mortality in children under five years, a significant cause of adult morbidity, and the leading cause of workdays lost due to illnesses. Malaria is the single most important infectious disease both in terms of admissions and as a cause of death in Ghana (Morrow 1984; Asenso-Okyere and Dzator 1997). Malaria and anaemia accounted for 41% and 18% respectively of hospital deaths in the Kassena-Nankana district of northern Ghana in1996 (Koram *et al.* 2000). Among pregnant women, malaria accounts for 13.8% of OPD attendance, 10.6% of admissions and 9.4% of deaths in Ghana (Marfo 2001).

2.2.3 Antimalarial drug resistance in Ghana

Antimalarial drugs are not regulated in Ghana, resulting in prescribing patterns and preferences differing greatly between areas and population groups. Chloroquine, amodiaquine and SP are commonly sold over the counter in shops and pharmacies throughout Ghana (Agyepong *et al.* 2002; Abuaku *et al.* 2004). In 1986, soon after Hogerziel and others (Hogerzeil *et al.* 1985), reported full sensitivity of *P. falciparum* to chloroquine in an *in vitro* test, Neequaye reported a case of resistance to chloroquine in a sickle cell patient seen at the Korle-Bu Teaching Hospital who had been on routine chloroquine prophylaxis (Neequaye 1986). Subsequently, in the same hospital, Neequaye and Ofori-Adjei in 1988, separately documented *P. falciparum* resistance *in vivo* at RI and RII levels in semi-immune children (Neequaye *et al.* 1988; Ofori-Adjei *et al.* 1988). Afari and others (Afari *et al.* 1992) conducted the first community studies to investigate the sensitivity of *P. falciparum* to chloroquine and other antimalarial drugs in Ghana in 1992 and 1993 in different ecological zones of Ghana. They observed resistance of *P. falciparum* to chloroquine to be greater in the coastal zones of Ghana compared to the forest zones. Afari and his team using the *in vivo* test, observed 91.1% sensitivity to chloroquine and 8.9% resistance response to chloroquine at RI (5.1%) and RII (3.8%) levels respectively. The distribution of resistance responses was about 17% to 23% in the coastal zone, 8.6% to 10% in the savannah zone and 3% to 6% in the forest zone. The RII responses occurred mainly in communities in the coastal zone. The investigators did not find any RIII resistance in any of the zones. The *in vitro* test showed resistance levels to be up to 60% and 4% in the coastal and forest zones respectively. Resistance to amodiaquine was recorded in 28.6% of the successful tests in the coastal zone. In 1994, Landgraf and others, in a peri-urban area of Accra that was one of the sites of the previous study by Afari and his team, found that resistance to chloroquine at the RI/RII level had risen to 45% among school children and, for the first time, reported 5 resistance responses at the RIII level (Landgraf *et al.* 1994). They also reported 37% resistance responses to SP at RII (84%) and RIII (16%) levels.

The Ghana Malaria Baseline Report of 2001 reported chloroquine efficacy tests carried out in six sentinel sites in Ghana (Marfo 2001). This showed that adequate clinical response to chloroquine ranged between 72% and 92%; early treatment failure ranged between 4% and 18% whilst late treatment failure ranged between 4% and 14%. The highest clinical response of 92% was recorded in the capital, Accra, whilst the lowest was in Sunyani (tropical rainforest zone). Parasitological sensitivity ranged between 50% (Tarkwa) and 78% (Accra). RI resistance ranged between 14% and 28%, the highest occurred in Navrongo whilst the lowest was recorded in Accra. RII resistance was between 4% and 8% whilst RIII was between 4% and 14%; the highest was recorded at Yendi. Overall, sensitivity to chloroquine was only 58%, RI resistance was 20%, and RII was 7% whilst RIII resistance was 8%.

The indirect cost (as computed by the amount of time spent in seeking care and taking care of the sick) per case of malaria is about 79% of the total cost of seeking treatment in Ghana. The estimated average cost of treating an episode of malaria including direct costs and the opportunity costs of travel and waiting time amounted to $8.67 or 3.7 days of male output or 4.7 days of female output (Asenso-Okyere and Dzator 1997).

2.2.4 Malaria control in Ghana

Malaria control efforts have been fragmented, uncoordinated, and in some cases antagonistic, rather than synergistic (Marfo 2001). This is because the planning and execution of malarial control activities in Ghana, which started in the pre-independence era just before the Second World War, have been undertaken mainly by the Ministry of Health sometimes to the exclusion of other stakeholders. Furthermore, various groups and organizations have consequently been working independently of each other on issues relevant to malaria control. With this background the Ministry of Health fully embraced the principles of RBM and has, since 1998, taken steps to follow the principles of RBM within the policy framework of

Ghana's Vision 2020 development plan. Aikins and Marfo in 1999 independently traced the historical developments in the control of malaria in Ghana (Aikins 1999; Marfo 1999).

2.2.4.1 Historical review of malaria control in Ghana

1920s

Malaria control activities in Ghana were initially restricted to a few urban areas covering about 20% of the country. These activities were aimed at malaria eradication and involved improvement of sanitation, institution of prophylactic measures and wide use of insecticides. There was construction of drains in urban areas aimed at eliminating stagnant waters and breeding sites of the mosquitoes. The World Economic Depression in 1930 caused the administration of quinine, proguanil and the twice a week pyrimethamine to schoolchildren in the country to be stopped.

1930s

Quinine was sold in post offices throughout the country with the intent of improving access to prophylactic care for the population in rural areas who had difficulty in accessing formal health services.

1945 to late 1950s

A malaria control unit of the Ministry of Health was set up in the Volta region in 1957 in collaboration with the WHO to oversee the malaria eradication programme. The focus of the eradication programme was environmental control to eliminate breeding sites. Larviciding agents such as kerosene and DDT and insecticides were introduced for domestic use.

1959 - 1963

A chemoprophylactic strategy of using chloroquine-treated salt through the Pinotti pilot project was adopted in some communities in northern Ghana and other parts of the country. This was abandoned because of the high operational cost involved.

1961 - 1968

The Ministry of Health with assistance from the WHO formed the National Malaria Service, headquartered at Ho in the Volta Region. This programme involved the training of many technicians and microscopists, which facilitated the conduction of malariometric and entomological research work on malaria in Ghana. Between 1964 and 1966 aerial spraying of mosquitoes was started together with treatment, on a large-scale, of permanent water bodies with diesel oil, DDT, dieldren or gamexane in Accra in addition to control activities in other parts of the country.

1968

Most of the above strategies did not achieve the desired impact because each phase relied on a single tool, rendering it ineffective. For example due to excessive use, resistance developed to DDT in some areas. Operational difficulties were experienced in maintaining these activities over extended periods. These problems were compounded by the diminishing sources of funding for the initiatives. By the end of 1967, it was realised that the malaria eradication strategies were not sustainable and so, in 1968, on the advice of WHO, malaria eradication campaigns were terminated in Ghana.

1970s and 1980s

Following the end of the malaria eradication campaign, malaria control received little support for two decades as there was a global perception that disease-specific approaches were ineffective.

1990s

A National Malaria Control Programme (NMCP) was formed in 1992, following the launch of the Global Malaria Control Strategy in 1992. The NMCP drew up a National Malaria Action Plan for the period 1993 to 1997 "to reduce the incidence of malaria to such low levels that it would cease to be a public health hazard in Ghana". This ambitious objective was set within the framework of the Global Malaria Control Strategy to prevent mortality,

reduce morbidity and the social and economic loss due to malaria. The objective was to be achieved through active case management, chemoprophylaxis and personal protective measures such as screening of houses and use of bed nets. Health education programmes of the Ministry of Health on television and radio supported these measures. However, due to a "top-to-bottom" approach to implementation, lack of financial and human resources and inadequate laboratory services this plan was not fully implemented.

In 1998, the Medium Term Strategic Plan for Malaria Control was drawn up to cover the period 1998 to 2001 with a general objective to "strengthen the development and implementation of sustainable malaria control in Ghana". The plan was drawn up in response to an objective of the Ministry of Health's Medium Term Health Strategic Plan (1998 – 2001) to reduce malaria case fatality rate from 24 to 8 in 2001 and increase bed net use from 24% to 80% by 2001 (MOH 1995).

In 1999, as part of the Medium Term Strategic Plan for malaria control a National Malaria Control Technical Committee of 12 experts was inaugurated to: -

i. Review the plans, preparation, and pace for the implementation of malaria control activities in the country.
ii. Advise the NMCP on collaboration with other interested organisations.
iii. Advise on any other developments in the implementation of the National Malaria Action Plan.

In 1999, Ghana signed up to the Roll Back Malaria initiative. The Ministry of Health accepted the principles of the RBM initiative as being in consonance with the goals of the ministry's Medium Term Health Strategic Plan (1998 – 2001) and committed itself to its implementation. The goals of the medium term health strategy were: -

i. To provide universal access to a basic package of health services.
ii. To improve the quality and efficiency of the health services.

iii. To foster linkages with other sectors to provide health care to the population.

Overall the Ghana RBM initiative emphasizes strengthening health services and making effective prevention and treatment strategies more widely available.

2.2.4.2 National Malaria Control Programme

The National Malaria Control Programme (NMCP) was set up in 1992 under the Parasitic Diseases Unit of the Disease Control Unit of the Ministry of Health and charged with planning, monitoring and evaluation of malaria control activities at the national level (Aikins 1999).

In response to the RBM initiative, the NMCP is currently implementing a malaria control strategy that involves multi and inter-sectoral partners working together on an agreed plan (Strategic Plan to Roll Back Malaria) to reduce death and illness due to malaria by 50% by the year 2010. This plan was developed following a long consultative process initiated by the NMCP in 2000 to sensitise and build consensus on malaria control among various stakeholders at all levels of the health services delivery system.

The goal of the RBM initiative in Ghana is to reduce malaria specific morbidity and mortality by 50% by the year 2010 through the

i. Promotion of multiple malaria prevention strategies.
ii. Improvement of malaria case management at all levels (from household to health facility).
iii. Commissioning of focused and evidence-based researches into malaria.
iv. Improvement of partnership between all stakeholders in health at all levels including the community.

2.2.4.3 National antimalarial drug policy

Until April 2005, chloroquine was the first line drug of choice in Ghana for treating uncomplicated malaria at all levels of the health system with SP or quinine as second line drugs. Chloroquine was also used for chemoprophylaxis in pregnant women. The policy recommended a full therapeutic dose of chloroquine for the pregnant woman on first contact and 300mg weekly throughout pregnancy at the antenatal clinic and then for 6 weeks postpartum. The current antimalarial drug policy recommends amodiaquine plus artesunate combination for uncomplicated malaria and quinine is the recommended drug of choice for managing severe malaria.

Current national malaria control policy in pregnancy in Ghana

Pregnant women with malaria in their second or third trimester are to be treated with quinine or amodiaquine plus artesunate. The treatment of choice for those in the first trimester is quinine. The Ministry of Health has also implemented the policy of intermittent preventive treatment of pregnant women using SP (SP-IPT) in Ghana. It is to be administered between 16 to 36 weeks of gestation with at least a month's interval between doses. The policy change was effected early this year in 20 selected districts while the following activities (Banda *et al.* 2004) to manage the policy change and implementation are currently going on in the country in order to scale up national implementation of the policy: -

i. Orientation workshops for health workers on the possible adverse affects of SP.
ii. Printing and distribution of Adverse Drug Reaction (ADR) reporting forms to clinics and hospitals for recording ADRs when they occur.
iii. Establishment of ADR management mechanisms whereby ADRs will be managed and the cost absorbed by an exemption package for pregnant women.
iv. Printing of new ANC cards, registers and posters to facilitate the education of the public, especially pregnant women on the SP-IPT implementation.

v. Negotiations with the Ministry of Finance and the donor community for SP to be purchased and made available to pregnant women free.

Some district hospitals in Ghana apart from the selected 20 including the St. Theresa's hospital have taken the initiative to implement the policy.

National mother-to-child HIV transmission control policy

There is no specific programme currently in Ghana to combat mother-to-child transmission of HIV other than the general HIV education that a mother-to-be, or any other person, can get from the health volunteer in their community. However, HIV testing and counselling is found at most district and regional hospitals including St. Theresa's.

The Ghana AIDS Commission has currently made antiretroviral therapy available in only 3 regions but plans to make it available at all regional hospitals, at an affordable rate, by December of this year.

At St. Theresa's Hospital, the Hope Association was formed in August 2000 with the help of two US Peace Corps Volunteers. It is a support group for people living with HIV/AIDS (PLWHAS) in Nkoranza District. Its aim is to bring together those testing HIV positive, as well as AIDS orphans and other vulnerable children to help them live positively. Its membership is made up of trained counsellors from the hospital and a group of volunteers with HIV/AIDS (PLWHAS).

The association has been conducting workshops for people living with HIV/AIDS both on nutrition and self-care, and for community volunteers on HIV transmission information, prevention, and home based care for PLWHAS. Over the years, the association has trained many community volunteers who go from house to house to educate other community members. It has set up a volunteer counselling and testing unit in the hospital. At the unit, a trained counsellor does the primary counselling and another member usually a PLWHAS, is responsible for moving laboratory documents back and forth to cut down on breaches of

confidentiality. The association receives supplementary funding and support from the Ghana AIDS Relief Fund (GARFUND) and the Catholic Relief Services (CRS) and, with this support, it is possible to supply monthly doses of vitamins and ration of food supplements to members with HIV/AIDS.

2.3 Malaria in pregnancy

2.3.1 Epidemiology

Malaria in pregnancy is a major public health concern in endemic countries where it is estimated that more than one billion women are at risk of malaria and liable to suffer from its consequences when pregnant (Menendez 1995). The pattern of malaria in pregnancy depends on a woman's pre-existing immunity, which is influenced by the local malaria transmission profile. It is also influenced by parity, gravidity, gestational age, age and HIV/AIDS (WHO 2004). *P. falciparum* and *P. vivax* are the most important causative agents in pregnant women living in malaria transmission areas with *P. falciparum* being more frequently associated with severe morbidity (Singh *et al.* 1996; Singh *et al.* 2001).

In high transmission areas, the incidence of falciparum malaria infection is higher in pregnant women than in the non-pregnant population (Brabin 1985; Singh *et al.* 1999). The risk of malaria in pregnancy is higher in young mothers of low gravidity and parity (Steketee *et al.* 1988; Brabin 1991; Brabin *et al.* 1999; Singh *et al.* 1999; Rogerson *et al.* 2000; Zhou *et al.* 2002). Zhou and others showed that, in Cameroon, young primigravidae are 2 to 3 times more at risk of malaria than multigravidae and about 3 times more at risk than older women (Zhou *et al.* 2002). Similarly, Verhoeff and others showed in Malawi that the prevalence of *P. falciparum* infection at a first antenatal visit was about three times higher in primigravidae than in multigravidae ($P < 0.001$) (Verhoeff *et al.* 1999). Saute and others in Mozambique, found younger age, lower parity and the second trimester of pregnancy to be associated with risk of

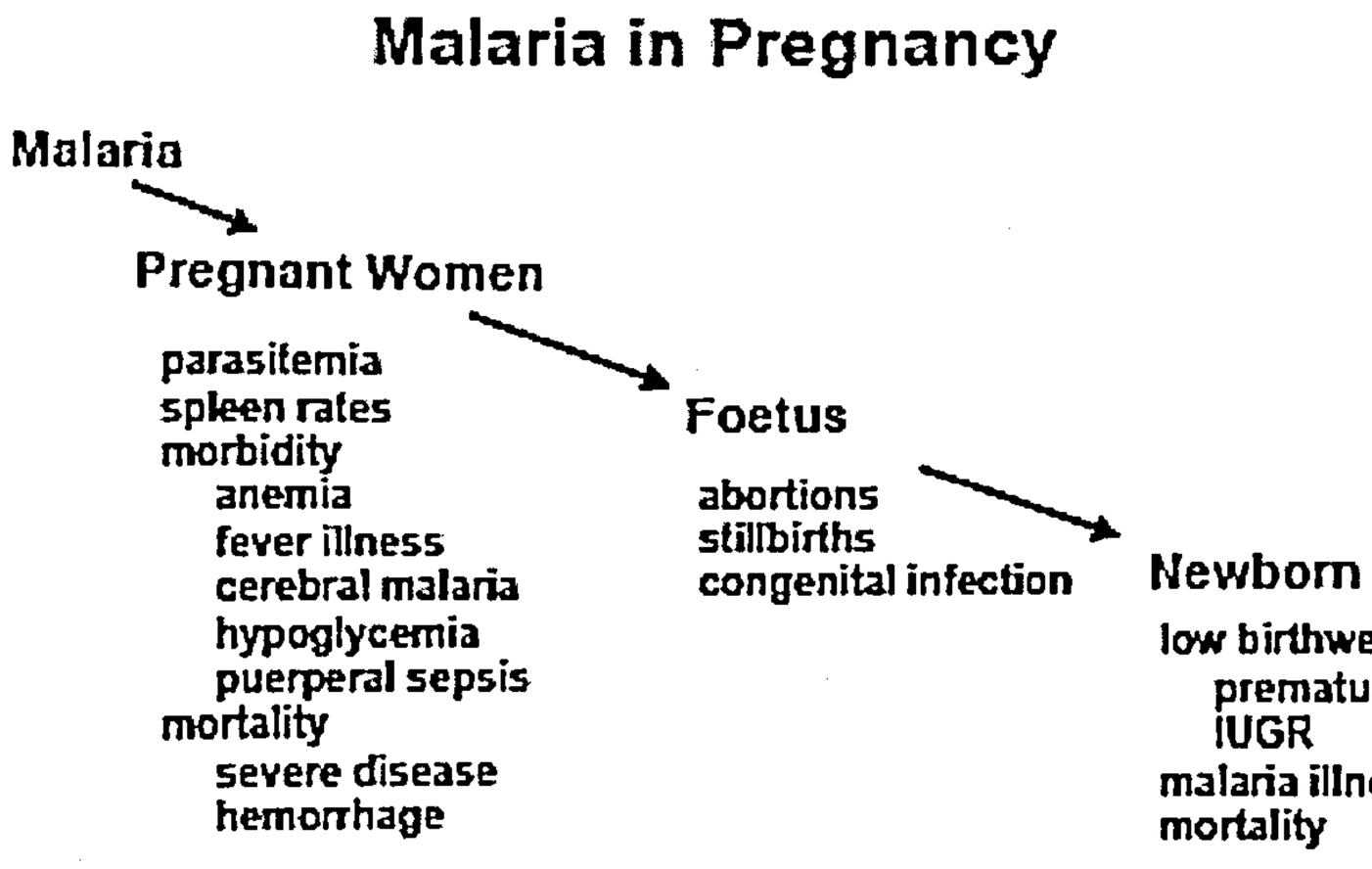
peripheral parasitaemia in pregnancy in a univariate analysis but only younger age remained significant when other factors were adjusted for (Saute *et al.* 2002).

HIV/AIDS and malaria in pregnancy interact adversely to increase the risk of severe maternal anaemia and low birth weight; treatments may not be effective and more doses may be required for SP-IPT (Steketee *et al.* 1996; Brabin 1997; Chandramohan and Greenwood 1998; Parise *et al.* 1998; Verhoeff *et al.* 1999; ter Kuile *et al.* 2004).

2.3.2 Pathogenesis

The pathogenic mechanisms of malaria in pregnancy are illustrated in **Figure 2.1** (WHO 2004). The complications of malaria in pregnancy result mainly from placenta parasitisation, maternal anaemia and the metabolic changes due to the infection. Pregnancy is associated with immunosupression due to reduced cell-mediated immunity and increased systemic levels of cortisol which make pregnant women more susceptible to infection.

Figure 2.1: - Pathogenic mechanisms of malaria in pregnancy.



With respect to malaria the loss of previously acquired immunity has been noticed to be more pronounced in primigravidae than in multigravidae. The mechanisms are not well understood but attempts have been made to explain the phenomenon as follows.

McGregor first postulated that the placenta is the preferred site for *P. falciparum* parasite sequestration and development which allows a parity specific immunity to be developed in the placenta. This immunity, however, does not protect first pregnancies but is retained in the uterus and gets stronger with subsequent pregnancies thus protecting them from placenta parasitaemia (McGregor 1984). Duffy and others showed that parasite sequestration in the placenta is brought about by distinct site-specific parasite variants or serotypes, which are able to adhere to glycosaminoglycans, for example chondroitin sulfate A (CSA) and hyaluronic acid (HA) that line the placental blood spaces. The adhesion process is mediated by the parasite protein PfEMP1, a variant protein, which is expressed on the surface of malaria-infected red blood cells. This leads to the accumulation of parasites and macrophages in the intervillous spaces which interfere with oxygen and nutrient supply to the foetus (Matteelli *et al.* 1997; Fried and Duffy 1998; Duffy and Fried 1999; O'Neil-Dunne *et al.* 2001; Duffy 2003).

Local immunity in the placenta is also thought to be suppressed by increased levels of placental oestrogen, which peak in the second trimester, and fall after that. This might explain the increased prevalence of malaria in the second trimester (Watkinson and Rushton 1983; Watkinson *et al.* 1985). The high circulating cortisol levels in pregnancy is thought to contribute to the increased susceptibility of pregnant women to malaria. Cortisol levels are highest in the primigravidae which may explain the increased risk and prevalence of malaria in primigravidaes (Vleugels *et al.* 1987; Vleugels *et al.* 1989; O'Neil-Dunne *et al.* 2001; Bouyou-Akotet *et al.* 2004).

Maternal anaemia is thought to be due to haemolysis and phagocytosis of uninfected erythrocytes and the increased autoimmune clearance of infected erythrocytes by the spleen (Fleming 1989; Mutabingwa 1994; Shulman *et al.* 1996).

The increased demands of the hyper catabolic state of malaria, the presence of a large parasite mass; the hypoglycaemic response to starvation and the increased response of pancreatic islets to secretory stimuli lead to hyperinsulinaemia and hypoglycaemia in pregnancy (Davis *et al.* 1994). The paroxysms of fever, anaemia, hypoglycaemia and placental insufficiency all contribute to the undesirable foetal outcomes of malaria-infected pregnancies which include abortion, still birth and low birth weight.

2.3.3 Clinical features

P. falciparum malaria in pregnancy is associated with deleterious consequences to the mother and foetus. Maternal anaemia, fever, hypoglycaemia, maternal and foetal death, abortion, still birth, intrauterine growth retardation and low birth weight are known complications of falciparum malaria (WHO 1998a). The importance of any of these depends on the local transmission profile in the pregnant woman's area of residence. In areas with stable and intense transmission, pregnant women generally remain asymptomatic despite sequestration of parasitized red blood cells in the placental microcirculation. This results in maternal anaemia and low birth weight, particularly in the primigravidae (Brabin 1985; Verhoeff *et al.* 1999; Shulman *et al.* 2001; Steketee *et al.* 2001). More than 80% of severe pregnancy anaemia (Hb < 7g/dl) is associated with malaria parasitaemia (Fleming 1989; Jackson *et al.* 1991; Ndyomugyenyi and Magnussen 1999) and 60% of cases of moderate anaemia in pregnancy (Hb = 7 – 11g/dl) are also associated with malaria in pregnancy (Fleming *et al.* 1986). Shulman and others showed that in Kenya, falciparum infection in primigravidae was strongly associated with moderate and severe anaemia. Severe anaemia was found to be more than twice as common in women with peripheral parasitaemia than in those who were not

parasitaemic, and parasitaemia was associated with a 2.2g/dl decrease in mean haemoglobin level (Shulman *et al.* 1996; Shulman *et al.* 2001). In their review, Guyatt et al (Guyatt and Snow 2001) showed that about half a million pregnant women develop severe anaemia as a result of infection with malaria in sub-Saharan Africa each year. It has been shown that the risk of low birth weight and maternal anaemia is three times higher in malarious zones compared to a non-malarious zones (Brabin and Piper 1997). Brabin and Piper (Brabin and Piper 1997) estimated that up to 40% of low birth weight babies born in malarious areas are attributable to malaria and less than 10% attributable to severe anaemia (Hb < 7.0 g/d). Guyatt and other estimate that malaria is responsible for 19% of low birth weight incidence in malaria endemic areas (Guyatt and Snow 2004).

In areas where the transmission is low and unstable, malaria in pregnancy is more symptomatic and acute affecting all parities equally. Maternal deaths and abortion may occur (Brabin 1985; Shulman *et al.* 2001; Adam *et al.* 2005)

2.3.4 Diagnosis of malaria in pregnancy

Antenatal diagnosis and treatment of malaria is one of the control measures recommended for pregnant women (WHO 2004). However, the diagnosis of malaria in pregnancy is a challenge in stable transmission areas. This is because antenatal malaria infection is often asymptomatic and parasitized red blood cells sequestrated in the placental microcirculation may not be detectable in peripheral blood. Anaemia, used as an indicator of malaria in pregnancy, may become recognisable only when it is severe (Fleming 1989; Brabin 1991; Shulman *et al.* 1996; Verhoeff *et al.* 1999; Shulman *et al.* 2001; Shulman *et al.* 2002). Some researchers have retrospectively diagnosed malaria in pregnancy from placenta blood or biopsy (Rasheed *et al.* 1992; Bulmer *et al.* 1993; Bulmer *et al.* 1993; Rasheed *et al.* 1993) but this is useful only for research studies.

In recent times malaria rapid diagnostic tests (RDTs) based on the immunochromatographic detection of specific parasite antigens in infected blood, have been developed. The RDTs in use currently detect either histidine-rich protein-2 (HRP-2) or plasmodium lactate dehydrogenase enzyme (pLDH) (WHO 1999b). Several studies evaluating either the HRP-2 based RDTs (Kilian *et al.* 1997; Tjitra *et al.* 1999; Singh and Valecha 2000; Tarimo *et al.* 2001; Forney *et al.* 2003) or pLDH based RDTs (Cooke *et al.* 1999; Piper *et al.* 1999; Iqbal *et al.* 2003; Palmer *et al.* 2003) have been published. However, only few studies (Leke *et al.* 1999; Mankhambo *et al.* 2002; Singer *et al.* 2004) have reported their use to diagnosis malaria at delivery and/or the presence of placental malaria. The OptiMAL® antigen test used in the present study utilises a dipstick coated with monoclonal antibodies against parasite lactate dehydrogenase. It is an intracellular metabolic enzyme and has isoforms which help in parasite species differentiation. The pLDH is produced only by live plasmodium parasites and so the OptiMAL® dipstick has the ability to differentiate live parasites from dead ones. They can distinguish *P. falciparum* from the non-*falciparum* species, but cannot distinguish between *P. vivax*, *P. ovale* and *P. malariae.* Since it detects circulating antigens, the OptiMAL® test may detect placental malaria that may be undetectable by microscopic examination of a peripheral blood smear (WHO 1999b).

On the contrary, diagnosis of malaria in pregnancy in low transmission areas is easier because the infection is often symptomatic and parasitaemia is detectable in peripheral blood.

2.3.5 Malaria control in pregnancy

Malaria in pregnancy is considered an emergency requiring prompt and effective treatment so clinical and parasitological failures are not acceptable (Shulman December, 2001). The essence of treating malaria in pregnancy in endemic regions is not only to achieve symptomatic relief but also to achieve full peripheral and placental parasite clearance so as to avert and/or control maternal anaemia and placental parasite sequestration, both of which

have deleterious effects on the foetus (Shulman December, 2001). However, the wide spread existence of chloroquine and SP resistance on the African continent makes this a difficult task (Schapira 1990). Treatment options now available for the treatment of malaria in pregnancy are limited due to safety concerns, affordability and supply. Because of these limitations, combinations of different control measures have been recommended by the WHO to reduce maternal infection (Shulman 1999; Shulman *et al.* 1999; Shulman *et al.* 2001; WHO 2004; Shulman December, 2001). These include, the early diagnosis and treatment of malaria, antimalarial intermittent preventive treatment during pregnancy, antimalarial chemoprophylaxis and protection from exposure to the bites of the vector (especially the use of insecticide treated materials including bed nets) (Garner and Brabin 1994; Shulman 1999; Shulman *et al.* 1999; Garner and Gulmezoglu 2000; Shulman *et al.* 2001).

2.3.5.1 Antimalarial chemoprophylaxis

Routine chemoprophylaxis using locally effective antimalarials has been shown to reduce antenatal parasitaemia, prevent the prevalence of severe maternal anaemia and the associated adverse pregnancy outcomes (Spencer *et al.* 1987; Ogunwande 1991; Cot *et al.* 1992; Nyirjesy *et al.* 1993; Cot *et al.* 1995). The beneficial effects of chemoprophylaxis on primigravidae appear to be more marked than in multigravidae (Greenwood *et al.* 1994; Greenwood *et al.* 1994; Cot *et al.* 1995). However, widespread low compliance, low efficacy of chloroquine, lack of awareness and adverse drug effects have caused problems for the widespread implementation of antimalarial chemoprophylaxis during pregnancy.

For example, a recent survey of 422 pregnant women in 5 communities in Ghana by the National Malarial Control Programme indicated that less than half of pregnant women took antimalarial chemoprophylaxis during pregnancy and only 11.6% of them took it appropriately (Marfo 2001). The rationale for weekly chloroquine prophylaxis has become weak as a result of wide spread resistance to this drug, thus reducing its efficacy. Steketee and

his colleagues (Steketee *et al.* 1996) showed the reduced efficacy of chloroquine on placental parasitaemia clearance compared to mefloquine in Malawi. Apart from parasite resistance, the adverse effects of chloroquine, such as pruritus, do not encourage prophylaxis with this drug. Also studies in Kenya and Nigeria have not found the use of pyrimethamine or chlorproguanil as single drugs for malaria prophylaxis in pregnancy to be effective because of the high prevalence of resistance (Nguyendinh *et al.* 1982; Spencer *et al.* 1986; Watkins *et al.* 1987; Nahlen *et al.* 1989).

2.3.5.2 Intermittent Preventive Treatment (IPT)

The WHO has recommended the use of intermittent preventive treatment with SP as one of the control measures for malaria in pregnancy in areas with stable malaria transmission and increasingly many countries in these regions are changing their national malaria control policies accordingly (WHO 2005). Intermittent preventive treatment with SP decreases parasitaemia and severe anaemia and improves birth weight in areas where *P. falciparum* is sensitive to this drug (Shulman *et al.* 1999). It reduced by half the incidence of low birth weight in primigravid and multigravid women who had been given two doses compared to that seen in subjects who had been given one. However, this effect of SP was greater in primigravid women (Verhoeff *et al.* 1998). In Malawi, Rogerson and others (Rogerson *et al.* 2000) showed that in pregnant women who received SP-IPT the prevalence of placenta malaria was decreased by a third and the prevalence of low birth weight decreased by a half compared to the prevalences in pregnant women who did not receive SP-IPT. In this population however, coverage with SP-IPT was only about 30%. The effectiveness of the SP-IPT policy therefore depends on *P. falciparum* sensitivity to SP and local antenatal coverage. The increasing resistance to SP and low local antenatal coverage may limit the effectiveness of SP-IPT. Holtz and others showed that in Blantyre, Malawi where utilization of antenatal services is near 100%, SP-IPT coverage is under 40% (Holtz *et al.* 2004).

2.3.5.3 Insecticide Impregnated Bed Nets (ITNs)

Although, the use of ITNs as a vector control measure in pregnancy is recommended (WHO 2004), results of assessments of the impact of impregnated bed nets are not consistent because of the complexity of factors that determine acquisition and continued use of the nets. Browne in Ghana (Browne *et al.* 2001) and Shulman in Kenya (Shulman *et al.* 1998) did not find any effect on malaria and anaemia in pregnancy following the use of insecticide impregnated bed nets. Dolan in 1993 however found a reduction in maternal anaemia with the use of ITNs in the mesoendemic area of the Thai-Burmese border (Dolan *et al.* 1993) and similarly D'Alessandro in The Gambia suggested that a reduction in percentage of premature babies might be due to the use of ITNs (D'Alessandro *et al.* 1996). Also results from a large, cluster randomised control trial in the Kisumu area of Kenya showed that the use of ITNs significantly reduced the prevalence of parasitaemia in pregnancy and the incidence of both maternal anaemia and low birth weight in the study population. The study showed that the use of ITNs had a 25% protective efficacy against peripheral and placenta parasitaemia in pregnant women. There was a 0.6g/dl increase in haemoglobin concentration at delivery for pregnant women who used ITNs and a significant reduction in the proportion of severe anaemia at delivery. Babies born to women who used ITNs weighed about 80 grams more at birth compared to the weight of babies born to mothers in the control groups. These beneficial impacts of ITNs were, however, significant mainly in women with 4 or less gravidities (ter Kuile *et al.* 2003).

2.4 Antimalarial drug use in pregnancy

Chloroquine and SP appear to be the safest, most readily available and affordable drugs for treating or preventing uncomplicated malaria in pregnancy in Africa south of the Sahara.

2.4.1 Chloroquine (CQ)

Chloroquine is a 4-aminoquinoline that has a good schizonticidal activity against all chloroquine-sensitive plasmodial infections. It is also gametocytocidal against *P. vivax, P. malariae* and *P. ovale* as well as active against immature gametocytes (stages1–3) of *P. falciparum* (WHO 2000c; Winstanley *et al.* 2004). Until *P. falciparum* resistance eroded confidence in its use (White 1998), chloroquine was the first-line drug of choice for treating uncomplicated malaria in most malaria endemic countries including Ghana. Pruritus is the most frequent adverse effect reported (Sowunmi *et al.* 1998; Taylor and White 2004) for chloroquine which may be severe enough to compromise compliance in some patients. Other adverse events reported include transient headaches, nausea, vomiting, gastrointestinal symptoms (Steketee *et al.* 1987; Steketee *et al.* 1996). Attacks of acute porphyria and psoriasis may be precipitated in susceptible individuals. Very rare adverse events include leucopoenia, bleaching of the hair and, extremely rare adverse events include aplastic blood and neurological disorders, such as polyneuritis, ototoxicity, seizures and neuromyopathy (Steffen *et al.* 1990). Lange and others did not find any evidence of retinopathy in long term chloroquine users (Lange *et al.* 1994). Its use in pregnancy is considered safe (Wolfe and Cordero 1985; Parke 1988; Luzzi and Peto 1993; Phillips-Howard and Wood 1996) with no increases in abortions, still births and congenital abnormality rates when administered at normal therapeutic doses.

2.4.2 *Amodiaquine (AQ)*

Amodiaquine is a 4-aminoquinoline antimalarial drug similar in structure and activity to chloroquine with antipyretic and anti-inflammatory properties (WHO 2000c; Winstanley *et al.* 2004).

Olliaro and others, in a review of published and unpublished studies conducted in Africa on the treatment of uncomplicated falciparum malaria, showed that amodiaquine is significantly more effective than chloroquine in clearing parasites, with a tendency for faster clinical recovery (Olliaro *et al.* 1996). Other reviews and studies (Muller *et al.* 1996; Mengesha and Makonnen 1999) including those of Brasseur and colleagues (Brasseur *et al.* 1999) in West and Central Africa respectively support this view. Compared to SP, amodiaquine cleared fever faster. With respect to parasite clearance, the two drugs were equally effective by day 7 (Brasseur *et al.* 1999).

After oral administration, amodiaquine is rapidly and extensively metabolised to desethylamodiaquine, the principal antimalarial entity (Winstanley *et al.* 2004) and may not be detectable after eight hours (Winstanley *et al.* 1990). Desethylamodiaquine is concentrated in erythrocytes and is slowly eliminated with a terminal elimination half-life of up to 18 days.

The most common adverse reactions to the therapeutic doses of amodiaquine used for malaria treatment include nausea, vomiting, abdominal pain, diarrhoea and itching (Brasseur *et al.* 1995; Sowunmi *et al.* 2001) . A less common effect is bradycardia (Ngouesse *et al.* 2001). Substantial attention has been focused on the possibility of serious liver toxicity in patients taking amodiaquine as chemoprophylaxis. Fortunately, such toxicity appears to be uncommon, but national malaria programmes do not recommend amodiaquine prophylaxis. It is not clear, according to the current literature, whether serious hepatotoxicity associated with amodiaquine is mediated by exacerbation of underlying liver disease or by direct actions of amodiaquine on the liver. When used for chemoprophylaxis, amodiaquine caused toxic hepatitis and fatal agranulocytosis (Hatton *et al.* 1986; Neftel *et al.* 1986). The incidence of

serious reactions in UK travellers using amodiaquine for prophylaxis was 1 in 1 700. Blood disorders occurred in 1 in 2 200 travellers and serious hepatic disorders in 1 in 15 650. Fatal events occurred in 1 in 15 500 travellers (Phillips-Howard and West 1990). The toxicity of amodiaquine seems to be related to the immunogenic properties of the quinoneimine produced by auto-oxidation of the parent drug (Winstanley 1990). Amodiaquine is therefore contraindicated in persons with known hypersensitivity to amodiaquine, in persons with hepatic disorders, and for chemoprophylaxis (Keystone 1990; Luzzi and Peto 1993). Jaeger et al reported that large doses of amodiaquine produce syncope, spasticity, convulsions and involuntary movements (Jaeger 1987). However, amodiaquine has been used extensively in Africa, especially in francophone Africa, for the treatment of malaria with few reports of serious adverse effects. Amodiaquine at the recommended doses does not have any teratogenic effects and there is no other evidence to contraindicate the use of amodiaquine for treatment of malaria during pregnancy (WHO 2000c).

Amodiaquine is administered over 3 days at total doses ranging between 25 mg and 35 mg of amodiaquine base per kg in dosage regimens similar to those for chloroquine. A regimen of 10 mg of amodiaquine base per day for 3 days (total dose 30 mg/kg) is recommended as it has the advantage of simplicity (WHO 2000c).

2.4.3 Sulphadoxine-pyrimethamine (SP)

SP is an antifolate combination drug, which acts by synergism of its two components – pyrimethamine and sulphadoxine against the parasite-specific enzymes, dihydropteroate synthetase and dihydrofolate reductase (Winstanley *et al.* 2004). It is an effective blood schizonticide against *P. falciparum* but less effective against other species of plasmodium. It does not show cross-resistance with the 4-aminoquinolines, mefloquine, quinine, halofantrine or the artemisinin derivatives (WHO 2000c). It has a long half-life, hence its formulation as a single dose drug, which is why compliance with it is high. SP has been used successfully in

areas with high *P. falciparum* resistance to chloroquine and during malaria epidemics. However, the long half-life of SP provides a potent selective pressure for parasite resistance in areas of high transmission.

There is a theoretical risk of jaundice among premature babies born to mothers given SP late in the third trimester. However, there is no evidence of increased risk of kernicterus in the newborn, (Anonymous 1983; Phillips-Howard and Wood 1996; Shulman *et al.* 1999) following exposure to antimalarial drugs containing sulphonamides or sulphones prior to delivery. The use of SP is also not associated with increased risk of adverse neonatal outcomes (Phillips-Howard and Wood 1996; Parise *et al.* 1998; Verhoeff *et al.* 1998). However, its use in the first trimester may be associated with birth defects (Hernandez-Diaz *et al.* 2000).

Therapeutic doses of SP for malaria are generally well tolerated. The most serious adverse reactions are associated with hypersensitivity to the sulphadoxine component, involving the skin and mucous membranes, usually occurring after repeated administration (Miller *et al.* 1986). Serious cutaneous reactions following single-dose treatment with SP are rare.

2.4.4 Amodiaquine/sulphadoxine-pyrimethamine (AQ/SP) Combination

The simultaneous use of two or more drugs as a chemotherapeutic strategy is already being employed in the treatment of leprosy, tuberculosis, cancer, and HIV/AIDS. The objective is to improve treatment outcomes and slow down the development of resistance to the individual drugs in the combination. Its application in malaria treatment involves the simultaneous administration of two or more blood schizonticidal antimalarial drugs either co-formulated or co-administered. The drugs should have a synergistic and not antagonistic anti-parasite effect when used together, should not interfere with each other's metabolism, should have a similar half-life and should not have any new adverse effects (WHO 2001b). Although

synergism is desirable between drugs in combination we are not aware of any pharmacokinetic studies of AQ+SP combination therapy in pregnant women or in children. However, by combining a shorter acting AQ with a slower acting SP it seems that a fast reduction in the parasite burden by AQ occurs which allows SP to act against the remaining diminished parasite burden. Hence the effect of AQ+SP on parasite burden may be additive. Antimalarial drug combinations have been used mostly in South-East Asia where multi-drug resistance is prevalent to improve treatment efficacy and to slow down the development of resistance to the component drugs (McGready *et al.* 1998; McGready *et al.* 2000; Nosten *et al.* 2000; van Vugt *et al.* 2000; McGready *et al.* 2001; McGready *et al.* 2003; McGready *et al.* 2005).

In West Africa, a change to AQ+SP is considered a possible option for combination therapy. AQ and SP have reasonably similar pharmacokinetic profiles, with varied modes of action on different biochemical targets in the parasite and are therefore technically suitable candidates for combination therapy. We are not aware of any studies reporting the use of AQ+SP combination in pregnant women either for treatment or for chemoprophylaxis. However, its use in children has been found to be safe and more efficacious compared to either AQ or SP alone (McIntosh and Greenwood 1998). A study in Uganda, reported a 100% adequate clinical response and a 99% parasitological success in children who received the AQ+SP combination compared to those who received SP alone or SP+CQ combination (Gasasira *et al.* 2003). In a multi-centre trial in Uganda, AQ+SP was found to be superior to CQ+SP combination in clearing parasitaemia and resolving symptoms in children (Bakyaita *et al.* 2005). Another study in Uganda also showed AQ+SP combination to be superior to AQ or SP alone in achieving adequate clinical and parasitological response following treatment uncomplicated malaria in children (Staedke *et al.* 2001). All the studies in Uganda showed the AQ+SP combination to be associated with more frequently with minor adverse effects. In Tanzanian children, the AQ+SP combination was found to be safe and had greater clinical and parasitological efficacy compared to AQ or SP alone (Schellenberg *et al.* 2002).

2.4.5 Mefloquine

Mefloquine is a 4-quinoline methanol chemically related to quinine. It is a potent long-acting blood schizonticide with a long elimination half-life and consequently gives long-lived sub therapeutic concentrations in the blood. It is highly active against *P. falciparum*, *P. vivax* and, *P. malariae* and most probably *P. ovale*. It is not gametocytocidal and is not active against the hepatic stages of malaria parasites. Mefloquine was used mainly in South East Asia to treat malaria resistant to both chloroquine and SP. However, since the late 1980s, resistance of P. falciparum to mefloquine has increased to more than 50% in 28 days after treatment with the drug. Mefloquine can be used both for therapy and chemoprophylaxis. Mefloquine is recommended as a prophylactic drug for travellers to areas with significant risk of chloroquine-resistant falciparum malaria.

The main disadvantage of mefloquine relates to the numerous adverse effects associated with its use either for treatment or for prophylaxis. These include dizziness, mild to moderate nausea, vomiting, diarrhoea and abdominal pain. In addition its use is associated with neuropsychiatric adverse reactions such as affective disorders, anxiety disorders, hallucinations, sleep disturbances including nightmares and, in a few people, overt psychosis, toxic encephalopathy, convulsions and acute brain syndrome (Corbett *et al.* 1996). Mefloquine use is also associated with bradycardia and sinus arrhythmia in up to 68% of patients treated in hospital-based studies. Other adverse reactions reported rarely include blood dyscrasias, transient elevation of transaminases, blackwater fever, Stevens-Johnson syndrome and toxic epidermal necrolysis. Concurrent use of quinine can potentiate dose-related adverse reactions to mefloquine and so mefloquine should not be administered within 12 h of the last dose of quinine. Co-administration of mefloquine with tetracyclines or ampicillin also produces higher mefloquine blood concentrations.

Mefloquine used for both chemoprophylaxis and treatment during the second and third trimesters of pregnancy is efficacious and safe (Nosten *et al.* 1994; Steketee *et al.* 1996; Adam

et al. 2004). However, its use during the first trimester may be associated with increased risk of abortion (Phillips-Howard *et al.* 1998) or stillbirths (Nosten *et al.* 1999). In non-pregnant women of childbearing potential, mefloquine can be prescribed for chemoprophylaxis, but pregnancy should preferably be avoided during and for 3 months after completing chemoprophylaxis. Mefloquine is excreted in breast milk in small amounts, the activity of which is unknown. Circumstantial evidence suggests that adverse effects do not occur in breastfed infants whose mothers are taking the drug (Bangchang *et al.* 1994).

2.4.6 Artemisinins

Artemisinin *(qinghaosu)* is an antimalarial isolated from *Artemisia annua* L. Artemisinin is poorly soluble in oils or water but the parent compound has yielded dihydroartemisinin, the oil soluble derivatives artemether and arteether, and the more water-soluble derivatives sodium artesunate and artelinic acid. These derivatives have more potent blood schizonticidal activity than the parent compound and are the most rapidly effective antimalarial drugs known. They are used for the treatment of severe and uncomplicated malaria. They are not hypnozoiticidal but gametocytocidal activity has been observed. The antimalarial activity of artemisinin and its derivatives is extremely rapid and most patients show clinical improvement within 1 to 3 days after treatment. However, like many other antimalarials the recrudescence rate is high when the drugs are used as monotherapy (WHO 1998b; WHO 2000c). In response, the WHO recommends that treatment policies for falciparum malaria in all countries experiencing resistance to monotherapies should be combination therapies, preferably those containing an artemisinin derivative (ACT - artemisinin-based combination therapy) (WHO 2001b).

Reports on the use of these drugs during pregnancy are limited. Available data from 124 pregnancies exposed to artemisinin products in the first trimester and 607 pregnancies exposed during the second or third trimester suggest that the use artemisinin in pregnancy

might be safe (WHO 2003a). However, experimental studies in animals showed that artemisinin compounds caused foetal resorption and morphological deformities in rats (WHO 2003a). Therefore the WHO recommends that artemisinin compounds may be used in the second and third trimesters when other treatments are considered unsuitable. But its use outside this period may be recommended only after careful consideration of the harm that could result from untreated malaria during pregnancy against the risk of a teratogenic effect that may result from its use (WHO 2003a).

2.5 Iron and folic acid supplementation

Malaria, iron deficiency, folate deficiency, haemoglobinopathies and HIV/AIDS have been identified by various studies as major causes of anaemia in Africa south of the Sahara. These are thought to interact in a vicious cycle of depressed immunity, infection and malnutrition (Fleming 1989; Shulman *et al.* 1996; van den Broek 1996; Mockenhaupt *et al.* 2000). Thus iron and folate supplementation in addition to effective treatment and prevention of malaria are recommended in pregnancy.

There is *in vitro* evidence (Watkins *et al.* 1985) and some clinical evidence (van Hensbroek *et al.* 1995) that folic acid, even in physiological doses, administered concurrently with SP, can antagonize the action of SP and thus reduce its efficacy. It was therefore suggested that folic acid supplements should be delayed for one week after SP treatment to avoid an inhibitory effect on antimalarial efficacy (WHO 1997). However, recent evidence from The Gambia showed that folic acid did not inhibit the action of SP used for IPT for in pregnant women (Mbaye *et al.* 2005 submitted).

2.6 Evaluating antimalarial drug efficacy and safety in pregnancy

2.6.1 Therapeutic efficacy indicators

The search for indicators of antimalarial drugs efficacy and how to measure them have engaged the attention of many scientists recently. The importance of any parameters and how they are measured vary from stable to unstable endemic regions, often depending on the local malaria transmission profiles and the drug resistance situation. The suitability of any of these for public health and for research purposes is not clearly defined. Each of these has some strengths and weaknesses with respect to the local malaria transmission profile (Phillips-Howard 1999).

In the absence of protocols for specific population groups, various modifications were made to the WHO in-vivo test protocol originally developed to assess parasitological and clinical efficacy of chloroquine in children (WHO 2001a) to suite the characteristics of other populations under study. The current protocol (WHO 2003b) permits these modifications. In most in-vivo studies in pregnant women, the level of parasitological failure including or excluding new infections was assessed to measure efficacy of treatments under consideration. What differed usually is the type of treatment and the follow up schedules. In south East Asia where multi-drug parasite resistance is wide spread and most treatment trials in pregnancy have been conducted, follow up schedules vary from 28 to 63 days depending on the half life the antimalarial drugs are being assessed (Nosten *et al.* 1993; McGready *et al.* 2000; Bounyasong 2001; McGready *et al.* 2001; McGready *et al.* 2001; McGready *et al.* 2005). In Africa, the maximum follow up time was 42 days to assess the efficacies of chloroquine, SP, chlorproguanil or dapsone (Steketee *et al.* 1987; Keuter *et al.* 1990; Sowunmi *et al.* 1998).

In contrast haematological recovery, decreased incidence of low birth weight and perinatal mortality were used increasingly to assess the clinical efficacy of antimalarials used either for chemoprophylaxis or IPT in pregnancy, most of which took place in Africa as shown in **Table 2.2.** Placental parasitaemia was rather often measured to assess its relationship with

low birth weight, maternal anaemia at delivery or parity. Sometimes it was used to assess the impact of chemoprophylactic or IPT programmes.

Table 2.2: - Parameters used to evaluate antimalarial drugs efficacy in pregnant women

Reference	Country	Study design/regimens	Parameter assessed
(Cot *et al.* 1992)	Burkina Faso	RCT; CQ chemoprophylaxis	Birth weight
(Greenwood *et al.* 1992)	The Gambia	CQ chemoprophylaxis	Birth weight
(Greenwood *et al.* 1994)	The Gambia	Maloprim chemoprophylaxis	Birth weight
(Greenwood *et al.* 1994)	The Gambia	CQ chemoprophylaxis	Birth weight
(Cot *et al.* 1995)	Cameroon	RCT; CQ chemoprophylaxis	Peripheral, birth weight and placental parasitaemia at delivery
(Shulman *et al.* 1999)	Kenya	RCT; SP-IPT	Peripheral parasitaemia & Hb
(Verhoeff *et al.* 1998)	Malawi	Cross-sectional; SP-IPT	Peripheral parasitaemia, birth weight & Hb
(Cot *et al.* 1998)	Cameroon & Burkina Faso	RCT; CQ chemoprophylaxis	Haematocrit
(Parise *et al.* 1998)	Kenya	SP case management & SP-IPT	Placental parasitaemia and birth weight
(Rogerson *et al.* 2000)	Malawi	Cross-sectional; SP-IPT	Peripheral and placental parasitaemia, birth weight & Hb
(Kayentao *et al.* 2005)	Mali	RCT; weekly CQ, CQ-IPT & SP-IPT	Hb, birth weight & placental parasitaemia

2.6.2 Safety indicators

Monitoring the safety of antimalarial drugs in pregnancy depends on the safety profiles of individual drugs and may be difficult because some of the post treatment symptoms and signs may be related to the underlying parasitaemia or the physiological changes occurring in pregnancy. Frequently studies monitored clinical signs and symptoms (Steketee *et al.* 1987; McGready *et al.* 2001; Challis *et al.* 2004; McGready *et al.* 2005) that emerged or worsened relative to pre-treatment baseline or adverse neonatal outcomes (Wolfe and Cordero 1985; Parke 1988; Levy *et al.* 1991; Nosten *et al.* 1994; McGready *et al.* 2001; McGready *et al.* 2005) such as teratogenicity, miscarriage rates, still birth rates and perinatal deaths to assess the safety of antimalarial drugs in pregnancy. Most clinical symptoms are predictable and dose dependent, based on the pharmacology of the drug involved. In contrast, most adverse neonatal outcomes cannot be predicted from known pharmacology of the drug involved. But the commonest independent predictor of ADRs is the number of concurrent medications being taken. Therefore drug interactions with other prescription medications, over-the counter preparations or herbal remedies also need to be considered when evaluating an adverse drug reaction (Eisenhauer 2002).

There is however, a paucity of data evaluating biochemical abnormalities due to antimalarial drugs in pregnancy. To evaluate the safety of CQ, AQ, SP and AQ+SP combination, symptoms and signs and laboratory data need to be monitored post treatment for patterns consistent with Steven Johnson's syndrome, hepatitis, haemolysis, anaemia, agranulocytosis, neutropaenia, miscarriage and kernicterus.

The above review has shown that malaria in pregnancy is a major threat to the life of the mother and her foetus particularly in the first time mothers. Fortunately, effective tools including insecticide treated bed nets, IPT and treatment of malaria illness for the prevention and control of the deleterious effects are available. What is worrying is that the utilization of these tools is low coupled with the wide spread drug resistant *P. falciparum* parasites. A

starting point towards addressing this problem is to begin systematic monitoring of antimalarial drugs in pregnant women for their efficacy and safety while improving and searching for the optimum ways of deploying the intervention tools currently available.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER 3 MATERIALS AND METHODS

3.1 Study overview

This was a randomised, double blind, prospective, comparative study of chloroquine versus amodiaquine and SP alone or their combination in the treatment of malaria infection in pregnancy. Prior to enrolment into the study all pregnant women were screened for eligibility according set inclusion criteria at the antenatal clinic sessions held at St. Theresa's Hospital between March 2003 and September 2004. Pregnant women who met all entry criteria were assigned randomly to CQ, AQ, SP or the AQ+SP combination treatment arms on day 0; when an enrolled pregnant woman took the first dose of the assigned treatment under direct observation by the recruitment team.

Each enrolled pregnant woman was followed until day 28 following the initial treatment or until one or more criteria for withdrawal from study were met as defined in section 3.12. Continuation of follow-up beyond day 28 was passive when the woman visited on scheduled days as specified in sections 3.7.2 and 3.7.3. The development of any adverse drug effects during active follow-up was classified and managed according to the definitions and guidelines listed under section 3.11.

3.2 Study objectives

3.2.1 General objective

To compare the efficacy, safety and tolerance of AQ, SP and the AQ+SP combination with those of CQ in the treatment of antenatal falciparum malaria infection in pregnancy.

3.2.2 Specific Objectives

Primary

To determine the effect of AQ, SP and the AQ+SP combination compared with CQ on the prevalence of peripheral parasitaemia on day 28 post treatment.

Secondary

1. To determine the effect of AQ, SP and the AQ+SP combination compared with CQ on the prevalence of peripheral parasitaemia on day 14 post treatment.
2. To assess the accuracy of the OptiMAL® antigen test for detecting peripheral parasitaemia compared to microscopy.
3. To compare the incidence of adverse events in the treatment groups.
4. To compare the effect of study drugs on maternal haemoglobin on 14 and 28 days post treatment, and at delivery.
5. To compare the effect of study drugs on peripheral and placental parasite densities at delivery.
6. To compare the effect of study drugs on birth weight at delivery.
7. To compare overall incidences of adverse pregnancy outcomes (abortion, stillbirth, congenital abnormality, prematurity and intrauterine deaths) in the study group to local rates obtained from St Theresa's Hospital's records.
8. To determine the prevalence of different symptoms in parasitaemic women in comparison to women without parasitaemia.

3.3 Methods

3.3.1 Study design

This was a four-arm randomised, double-blinded clinical trial of the efficacy and safety of AQ, SP and the AQ+SP combination compared with chloroquine in the treatment of pregnant women with malaria parasitaemia. All study pregnant women received daily iron and folic acid supplementation.

3.3.2 Study location

The study was done at St. Theresa's Hospital in the Nkoranza district of Ghana. The Nkoranza district is one of thirteen administrative districts of the Brong Ahafo Region of Ghana. It occupies a surface area of 2,300 square kilometres. The district's population was 145,000 in 2002 with an annual growth rate of 2.6%. The population is a mixture of Brongs, Akans and people from other parts of the country. The district lies in the transition zone between the rain forest and the savannah regions of Ghana and it is a malaria endemic area. Illiteracy is high and, despite brisk commercial activities, poverty levels are also high. There are 13 health facilities in the district. These include St. Theresa's Hospital, 2 health centres, 9 functioning rural clinics and a private maternity home. St. Theresa's Hospital is owned and administered by the Catholic diocese of Sunyani. It functions as the district hospital through a collaborative arrangement between the diocese and the Ministry of Health of Ghana. It has a bed capacity of 80 and provides all basic medical services including adult medicine, paediatrics, surgery and obstetrics and gynaecology. It runs two antenatal clinic sessions a week and registers an average of 212 new clients per month. The hospital operates the government's policy of providing free basic antenatal care for pregnant women. The average number of antenatal visits by each pregnant woman is 3. This policy was, however, discontinued during the last quarter of 2002 because government severely delayed reimbursement of funds to hospitals. This basic care consists of free consultation, free

ferrous sulphate and folic acid, free urine protein and haemoglobin level determinations and free chloroquine prophylaxis. The prevalence of malaria parasitaemia in children in the district and neighbouring districts was found to be 46% (Yeboah Antwi *et al.* 2001). Malaria, for decades, has been the number one outpatient and inpatient diagnosis in the hospital and the most frequent cause of mortality in children in the district. Between 1997 and 2001 a total of 2135 pregnant women were diagnosed clinically as having malaria with 15% needing admission. Malaria is the cause of 21% of maternal admissions to the St. Theresa's Hospital and 20% of pregnant women attending the antenatal clinic has *P. falciparum* parasitaemia (H. Tagbor, unpublished data, 2002). Chloroquine was the drug of first choice for treatment of malaria in pregnancy in the hospital before this trial. Second line drugs used include SP and artesunate. The district is the site for the Safe Motherhood Initiative programme run by MaterCare International with funding from CIDA and also one of the field sites for the Vitamin A project of the Kintampo Health Research Centre. The health centres and clinics in the district also provide antenatal services.

3.3.3 Study population

The study population comprised pregnant women of all parities with a gestational age of 16 weeks and above who attended antenatal clinic services at St. Theresa's Hospital's between March 2003 and September 2004.

3.3.4 Pre-project activities

The project was planned to start in November 2002 and run for 18 months but started in March 2003 instead. The delay was caused by problems in the production the study drugs by Kinapharma Limited.

3.3.4.1 Project team

The PI in collaboration with the hospital management team recruited a core staff shown in **Table 3.1**, with varying educational background for the screening, fieldwork and data management activities of the project. In addition the project co-opted hospital staff from the laboratory, maternity wing and antenatal clinic of the hospital to help.

Table 3.1: Core Project Staff

Name	Educational level/Qualification	Role on project	Duration on project
Mark Asante	Higher National Diploma	Field Supervisor	24 months
Mark Sarkordie	Higher National Diploma	Data Entry Clerk	18 months
Godfred Owusu Ansah	Senior Sec. School Certificate	Data Entry Clerk	24 months
Ernest Butsorme	Higher National Diploma	Field Worker	24 months
Hannah Ampofowaa	Senior Sec. School Certificate	Field Worker	24 months
Florence Twumwaa	Senior Sec. School Certificate	Field Worker	24 months
Philip Akpagonu	Senior Sec. School Certificate	Field Worker	18 months
Kaizer Appiah-Kubi	Senior Sec. School Certificate	Field Worker	6 months
William Baffoe	Higher National Diploma	Field Worker	6 months
Pius Occlo	Higher National Diploma	Laboratory Technician	18 months
Francis Owusu Ansah	Higher National Diploma	Laboratory Technician	24 months

3.3.4.2 Project staff training

A one-month practical course in the topics listed below was organised for the field teams and data management team in order for them to understand the nature of the project and acquire the knowledge and skills needed for their work. Areas covered in training sessions included:

i. Malaria antigen test with OptiMAL® dipsticks
ii. Slide preparation and staining
iii. Venipuncture
iv. Haemoglobin concentration determination using Hemocue analysers
v. Detection of chloroquine and SP in urine using ELISA dipsticks
vi. Filter paper blood spot preparation
vii. Establishing rapport and interacting with clients

viii. Information gathering

ix. Motor bicycle riding

x. Basic computing and data entry

Staff were each assigned roles and responsibilities on the project according to the standard operating procedures. A clinician was responsible for performing obstetric ultrasound examination on all recruited pregnant women and received and monitored reports of adverse effects in the absence of the PI. Midwives were also trained to prepare slides for microscopy and determine haemoglobin concentration at delivery and record relevant data for the project. Antenatal staff were involved in educating pregnant women on the project during clinic sessions and liaised between the screening and recruiting teams during these sessions.

3.3.4.3 Sourcing test drugs

A Ghanaian pharmaceutical company Kinapharma Limited was contracted to manufacture and pack test drugs according to custom design for the project. The Food and Drugs Board of Ghana tested the tablets and confirmed their quality. Furthermore the drugs were also assayed at the London School of Hygiene and Tropical Medicine and found to be of standard quality.

3.3.4.4 Data and safety monitoring board

A data and safety monitoring board (DSMB) was constituted for the project. It had Prof. Bernard Brabin as chairman and Prof. Aggrey Oloo, Dr. Kalifa Bojang, Dr. Joanna Schellenberg, Dr. George Bonsu and Dr. Todd as members.

The board was responsible for: -

i. Regular monitoring of the data and safety issues concerned with the study.

ii. Reviewing the PI's reports on serious adverse events and making recommendations on further progress of the study.

iii. Reviewing the statistical analysis plan.

3.3.5 Inclusion and Exclusion Criteria

3.3.5.1 Inclusion Criteria

A pregnant woman was eligible for inclusion in the study if: -

1. Her pregnancy was at least 16 weeks.
2. She had *P. falciparum* parasitaemia of any density with or without symptoms.
3. She was willing to participate and complete the test schedule and had given informed consent.
4. She had no known adverse reaction to any of the study drugs.
5. She was willing to have supervised delivery at any maternity unit in the district.
6. She lived within the Nkoranza district.

3.3.5.2 Exclusion Criteria

A pregnant woman was ineligible for inclusion in the study if: -

1. She was less than 16 weeks pregnant.
2. She was not resident in the study area.
3. She had a past obstetric or medical history that might adversely affect the interpretation of outcomes of the trial such as repeated stillbirths and eclampsia.
4. She declined to participate.
5. She had a history of severe adverse drug reactions to co-trimoxazole in the past.
6. She had a haemoglobin concentration below 5.0 g/dl.
7. She had malaria that was severe enough to require parenteral medication.

3.4 Outcome measures

3.4.1 Primary

Prevalence of parasitaemia on day 28 post treatment defined as: -

a) Any level of peripheral parasitaemia by microscopy at day 28 post treatment.

b) Any level of peripheral parasitaemia by microscopy at day 28 post treatment excluding any new infections identified by MSP2 (merozoite surface protein-2) genotyping.

3.4.2 Secondary

1. Prevalence of parasitaemia on day 14 post treatment.

    a) Any level of peripheral parasitaemia by microscopy at day 14 post treatment excluding any new infection identified by MSP2 genotyping.

    b) Any level of peripheral parasitaemia by microscopy at day 14 post treatment.

2. Sensitivity, specificity, positive and negative predictive values, likelihood ratios, and the area under receiver operating characteristic (ROC) curve for the OptiMAL® antigen test.
3. Incidence of adverse drug events within seven days following treatment.
4. Proportions of pregnant women withdrawn from the study due to the occurrence of adverse drug events (clinical and laboratory) by day 7 following initiation of treatment.
5. Change in maternal haemoglobin concentrations at days 14 and 28 following treatment.
6. Prevalence of peripheral parasitaemia at delivery.
7. Prevalence of placental parasitaemia at delivery.

8. Proportions of abnormal biochemistry and white blood cell values on days 14 and 28 post treatment.
9. Incidences of adverse pregnancy outcomes in the study group.
10. Prevalence of postpartum parasitaemia.
11. Prevalence of postpartum anaemia.

3.5 Sample Size Calculation

The study sample size was based on the need to detect, with 95% confidence and 90% power, a reduction in the proportion of parasitological failures on day 28 in AQ, SP and AQ+SP versus CQ using the formula below.

$$n = \frac{[(z_1+z_2)^2\, 2P(1-P)]}{(p_1-p_2)^2}$$

Where:

n is the number of study pregnant women required in each treatment arm.

p_1 and p_2 are estimates of the proportions of failures in parasitological clearance in the chloroquine and other groups respectively after treatment.

P is the average of p_1 and p_2

z_1 is the 2-sided significance level probability of concluding that there is a significant difference in the observed proportions in the two groups when in fact there is no difference and corresponds to 1.96 (type I error)

z_2 is the one-sided probability of concluding that there is no significant difference in the observed proportions in the two groups when in fact there is a difference and corresponds to 1.28 (type II error)

In calculating the sample size, consideration was given to both statistical demands and practical feasibility. In the absence of recent local data on the level of falciparum resistance to

amodiaquine and SP at the design stage of this study, the sample size was calculated assuming various levels of resistance to these drugs, fixing the study power with an estimated failure rate of 22% (Marfo 2001) for chloroquine against uncomplicated falciparum malaria.

It was assumed that parasitological resistance to AQ, SP and AQ+SP combination would range between 5% and 15% against uncomplicated falciparum malaria in Ghana. The study was not powered to show a difference in efficacy between AQ, SP and the AQ+SP combination.

Table 3.2: Distribution of study sub sample according to treatment arm.

AQ, SP or AQ+SP failure rate	Fixed study power of 90%, CQ failure rate of 22%. Sample Size (N)		
	CQ/SP	CQ/AQ	CQ/AQ+SP
5%	98	98	98
8%	157	157	157
10%	225	225	225
12%	341	341	341
15%	743	743	743

1. These figures representing the sample sizes were adjusted to account for a 15% loss to follow up.

2. The study required between 98 and 743 study pregnant women in each treatment arm to give it a power of 90% to detect the difference between the 22% failure rate anticipated in the chloroquine group and failure rates of AQ, SP and AQ+SP combination in the range of 5% to15%.

3. Since chloroquine was the comparator drug in the 3 segments, a sample size of 225 pregnant women was considered adequate in each of the 4 arms giving a total study size of 900 pregnant women. This was based on the assumption of a 28-day

parasitological clearance of 90% for AQ, SP and AQ+SP combination, and 78% for CQ (α = 5% power = 90%).

3.6 Screening and enrolment procedures

All study pregnant women were recruited from the antenatal clinic of St. Theresa's Hospital over a period of 18 months. The principal investigator (PI), supported by midwives at the antenatal clinic conducted the recruitment during clinic hours. The standard operating procedures at the clinic were as follows:

1. All pregnant women who attended the antenatal clinic were screened initially for falciparum antigens using the OptiMAL® individual malaria test kit. Thin and thick blood films on the same slide were made at this point from the finger prick blood sample for malaria parasite identification and quantification. Women in whom the antigen test was positive and who satisfied the inclusion criteria were then taken through the next enrolment stages.
2. Informed consent was obtained from pregnant women who satisfied all inclusion criteria.
3. Pregnant women who tested positive for malaria with the dipstick test were assessed clinically and obstetrically with the view to enrolling them into the study. Information on their demographic and socio-economic characteristics, age and parity were obtained and recorded. Gestational age was confirmed with ultrasound scanning by a study clinician or the principal investigator.
4. Screening staff then obtained 5mls of venous blood from the antecubital vein for baseline investigations including haemoglobin level, filter paper blood spots, white blood cell counts (total and differential) and liver function tests (total bilirubin, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase).

5. All antigen positive thin and thick blood films were examined plus 5 randomly selected antigen negative slides.
6. Pregnant women whose antigen tests were negative went through the routine antenatal clinic procedures. Those whose were antigen positive but had negative microscopy or gametocytes only were treated with chloroquine according to national antimalarial treatment policy but not included in the study.
7. Randomisation and assignment of recruited pregnant women to treatment groups then followed.

3.6.1 OptiMAL® dipstick assay

The OptiMAL® individual rapid malaria test kits were bought from the manufacturers (Flow Inc.). The rapid malaria diagnostic dipstick assay was used to help to identify potential participants quickly as blood films could not be read immediately. The assays were performed and interpreted at the ANC by the screening team. Laboratory technicians who read blood films were blinded to the results of the assay. The assays were performed according to the manufacturer's instructions outlined below.

Each test kit has a main device constituted by a dipstick, conjugate and wash wells. Also in the package is a well cover, a buffer in a dropper, a pipette, and a disinfecting swab. The test proceeds, in five simple steps, as follows: -

Step 1

The device is positioned on a flat surfaced table. One drop of buffer is added to the conjugate well and four drops of the buffer to the wash well and the test allowed to stand for a minute.

Step 2

One drop of finger-prick blood (equivalent to 10 micro litre of blood) is added to the conjugate well, mixed gently and then allowed to stand for a minute.

Step 3

The dipstick is placed in the conjugate well and allowed to stand for 10 minutes.

Step 4

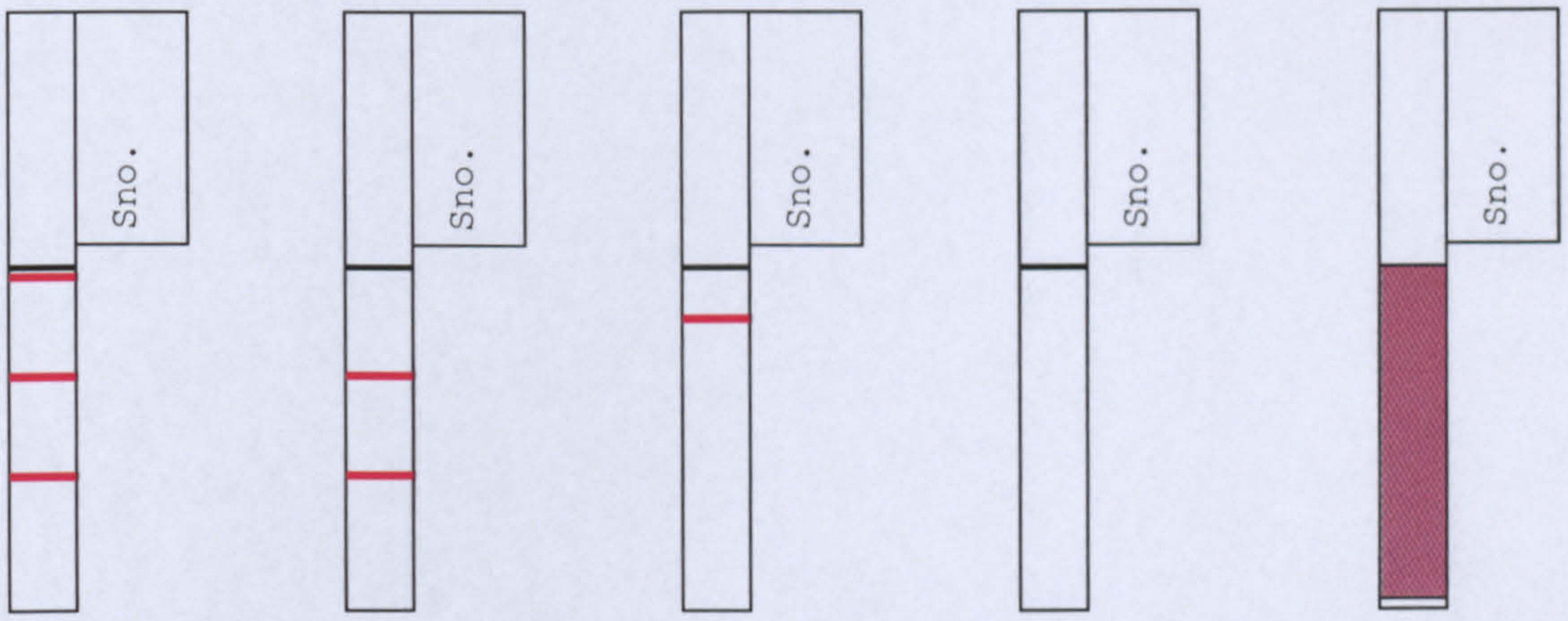
The dipstick is transferred to the wash well and allowed to wash for another 10 minutes.

Step 5

The reaction bands on the dipsticks are read and interpreted as shown in **Figure 3.1.**

- Two test bands and one control band indicate a *P. falciparum* infection.
- One test band and one control band indicate a *P. vivax* infection.
- One control band only at the top of the test strip is regarded as negative.

Figure 3.1: Reaction bands of OptiMAL® tests



Positive for	Positive for	Negative	Invalid	Invalid
P. falciparum	*P. vivax*/*P. malariae* *P. ovale*			

Figure 3.2: - A picture of an OptiMAL® dipstick screening session at the antenatal clinic.

For the purposes of estimating the level of *P. falciparum* parasitaemia, the intensity of positive reaction bands was graded strong, medium and weak as shown **Figure 3.3**.

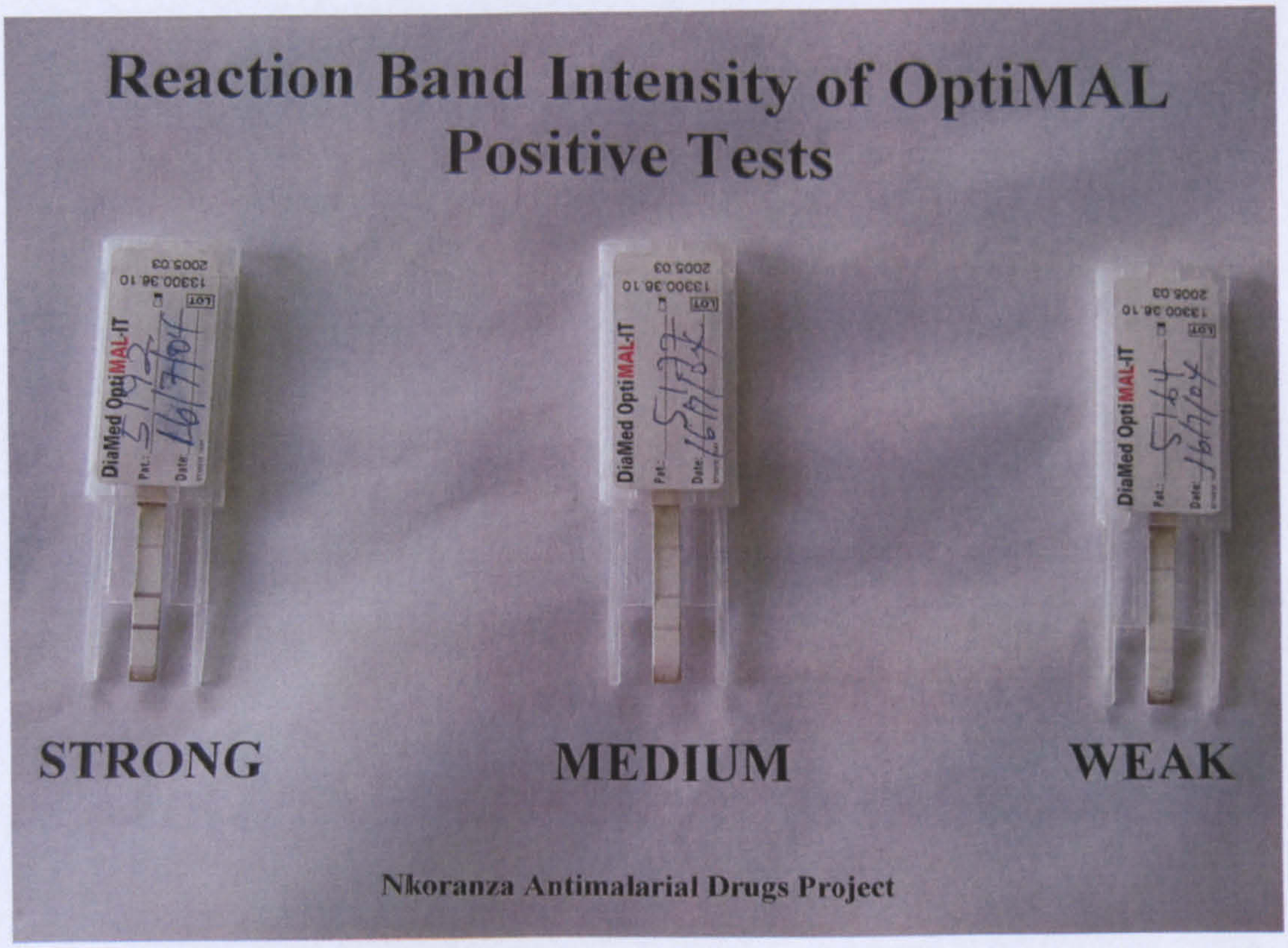


Figure 3.3: Reaction band intensities of positive test.

3.6.2 Clinical and obstetric assessment

On the day of recruitment, a detailed medical history, including obstetric and drug histories was obtained from every pregnant woman who had satisfied the inclusion criteria. Information on baseline characteristics such as age, height, occupation, educational status, marital status and address was obtained and recorded. The address detailed house number, if any, landmarks and contact persons to facilitate follow-up. This was followed by a general examination to record the axillary temperature to one decimal point in degrees centigrade using an electronic thermometer, a check for pallor and measurement of weight in kilogram using the Scala™ weighing scale. An obstetric examination was then conducted to confirm pregnancy and predict gestational age by symphysio-fundal height measurements and also by taking the last menstrual period into account. An ultrasound examination was performed on each woman to confirm gestation, gestational age and the viability of the pregnancy. A case recording forms were used to record information on each pregnant woman. These assessments were performed on each subsequent antenatal visit until delivery.

3.6.3 Laboratory tests

Apart from the biochemistry tests and the polymerase chain reaction assays, all other laboratory tests were carried out at the St. Theresa's Hospital.

3.6.3.1 Microscopic Blood Examination

Giemsa stain

A new working Giemsa solution was prepared for each staining session. For each session, two separate working solutions at pH of 7.2 were prepared with concentrations of 3% and 10% respectively.

Slide preparation and reading

Thin and thick blood smears were prepared for each antigen positive pregnant woman during each antenatal session. All slides were labelled with the respective identification number using a 'permanent' glass-writing pen to facilitate identification while ensuring blinding of microscopists to treatment allocations. Women who tested antigen positive had their smears stained; blood films for 5 randomly selected slides of antigen negative women were stained and examined on each clinic day. All slides were air-dried for 30 minutes in a netted box to protect them from flies. The slides were then stained for 10-15minutes with 10% Giemsa stain and an initial reading was made to determine the presence or absence of parasitaemia so that treatment could be administered. A second examination of each slide was made to count the number of parasites per 100 high power fields (HPF). This was used to calculate the parasite density (the number of parasites per micro litre of blood). One microscopist at a time examined the slides and estimated parasite counts as follows: -

i. Selection of a part of the thick film where the white blood cells (WBC) were evenly distributed and the parasites well stained.
ii. Systematic counting of 200 or more white blood cells and estimation at the same time of the number of asexual parasites in each field using two, hand-tally counters.
iii. The parasite density was calculated using the formula (WHO 1994; GILLES and WARRELL 1996; Cheesborough 1998):

$$\text{Parasite Density} = \frac{\text{Number of parasites} \times 8000}{\text{Number of white blood cells}}$$

If more than 500 parasites had been counted without having reached 200 leucocytes, the count was stopped after completing the reading of the last field, and the parasite count calculated according to the formula above. When the number of asexual parasites counted

against 200 leucocytes was below 10 parasites, counting was done against 500 or more leucocytes. A blood slide was pronounced negative only when examination of 100 HPF of a thick film did not show the presence of asexual forms of *P. falciparum*.

The same techniques were employed for establishing parasite counts on each of the subsequent blood films prepared on subsequent follow-up visits.

Independent quality assessments

The Noguchi Memorial Institute of Medical Research (NMIMR) acted as an independent agency for parasitological quality assurance assessment of the Nkoranza Antimalarial Drugs Project. Five hundred and forty-seven slides from the project were randomly selected and submitted for quality assurance testing by a microscopist (Mr. Charles Atiogbe) at NMIMR.

Interobserver variability in the interpretation of microscopy results by microscopists from the St. Theresa's Hospital and Noguchi Memorial Institute of Medical Research respectively were generally identical. The details of this are shown in **Appendix 15**. All the negative slides sent were confirmed negative at NMIMR. The day 0 sample included 81 positive slides of women recruited into the study but 6 of them were read negative by the microscopist at NMIMR. Forty-nine day 7 slides were re-assessed but it was realised later that the sample had only one positive slide.

3.6.3.2 Polymerase chain reaction (PCR) assays

Polymerase Chain Reaction (PCR) assays on filter paper blood spots obtained from the field were carried out in the London School of Hygiene and Tropical Medicine by Rosalynn Ord and Anna Randall using their laboratory protocols based on methods described for DNA extraction from blood spots (Plowe *et al.* 1995) and msp2 genotyping (Snounou *et al.* 1999). Briefly, the repetitive polymorphic loci, block 3 of *msp2* (merozoite surface protein 2), was amplified by nested-PCR after DNA had been extracted from filter paper blood spots using

the chelex extraction method. In the nest 1 reaction, amplification was performed on a total reaction volume of 30µl containing 16.8µl of nuclease free water, 3µl of KCl buffer, 3µl of dNTPs, 1µl of M2-OF, 1µl of M2-OR, 0.2µl of Taq and 5µl of DNA extract each. The nest 1 product was then used as the template for the nest 2 reaction.

In the nest 2 reaction, amplification was performed on a total reaction volume of 32µl containing 21.4 of nuclease free water, 3.2µl of KCl buffer, 3.2µl of dNTPs 1µl of forward primer F', 1µl of Reverse primer R' 0.2µl of Taq and 2µl of nest1 product each. The nest 2 amplified products were then electrophoresed on a 2.5% agarose gel. Ethidium bromide was used to stain the PCR product following electrophoresis on agarose gel in Tris-Borate EDTA (TBE) buffer and the results were visualised using ultraviolet light. The number of distinct amplification bands for both allelic variants was counted to estimate of the minimum number of genotypes present in each sample. The bands were then compared between day 0 and treatment failure day pairs. If the bands were the same, the sample was considered a true treatment failure. The presence of new alleles indicated new or re-infections. If a band could not be classified as above it was labelled indeterminate.

3.6.3.3 Haemoglobin measurements

At recruitment the haemoglobin levels were measured using a Hemocue™ analyser for each woman according to the manufacturer's instructions. The measurements were repeated on days 14 and 28 after treatment and at delivery and six weeks postpartum.

3.6.3.4 White blood cells counting

White blood cells for women in the study were counted microscopically using uncoagulated whole blood diluted with a WBC diluting fluid (a staining weak acid solution), the Improved Neubauer ruled counting chamber and a manual differential tally counter. The total WBC

count was obtained by dividing the total number of cells counted by 20 and multiplying the result by 10^9. The individual white cells were expressed as fractions of the total.

3.6.3.5 Biochemical assessments

Frozen sera were transported on ice weekly to the Clinical Analysis Laboratory of the Biochemistry Department of the University of Science and Technology, Kumasi for all biochemistry tests.

Liver Function Tests

Commercial reagent test kits were used for the liver function tests. For AST and ALT, individual kits manufactured by RANDOX laboratories Ltd (Diamond Road, Crumlin, Co. Antrim, UK) were used while kits manufactured by BIOLABO (S.A. France) were used for bilirubin and GGT determination. The tests were performed according to the manufacturers' instructions detailed below.

Aspartate aminotransferase (AST) determination

In the presence of AST, aspartate is deaminated to form oxaloacetate, which reacts with 2, 4-dinitrophenyhydrazine (DNPH) a colour reagent to form a brown oxaloacetate hydrazone solution, which is measured spectrophotometrically.

$$\alpha\text{-oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{Oxaloacetate}$$

Procedure

The test kit contains two vials; vial 1 contains an already made reagent constituted by phosphate buffer, L-aspartate and α-oxoglutarate and vial 2 contains DNPH. Five hundred micro litres of the content of vial 1 was pipetted into test tubes labelled "reagent blank" and "sample". This was followed by the addition of 100μL of distilled water into the reagent

blank tube and the 100μL of serum into the sample test tube. The resulting solutions were well mixed and incubated at 37°C for 30 minutes.

Five hundred micro litres of DNPH was added into each tube, mixed and allowed to stand for exactly 20 minutes at room temperature. Five hundred micro litres of sodium hydroxide was then added to each tube and the absorbance of the test sample read at a wavelength of 546nm against the reagent blank. The concentration of the AST in the serum was obtained from **Table 3.3.** The reference range for this kit is values up to 12 U/L.

Table 3.3: - Range of absorbance and corresponding AST activity

Absorbance Range	U/L	Absorbance Range	U/L
0.020 – 0.029	7	0.100 – 0.109	36
0.030 – 0.039	10	0.110 – 0.119	41
0.040 – 0.049	13	0.120 – 0.129	47
0.050 – 0.059	16	0.130 – 0.139	52
0.060 – 0.069	19	0.140 – 0.149	59
0.070 – 0.079	23	0.150 – 0.159	67
0.080 – 0.089	27	0.160 – 0.169	76
0.090 – 0.099	31	0.170 – 0.179	89

Alanine aminotransferase (ALT) determination

In the presence of ALT, alanine is deaminated to form oxaloacetate, which reacts with DNPH to form a brown oxaloacetate hydrazone solution, which is measured spectrophotometrically.

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate} + \text{Pyruvate}$$

Procedure

The test kit contains two vials; vial 1 contains an already made reagent constituted by phosphate buffer, L-alanine and α-oxoglutarate and vial 2 contains DNPH. Five hundred

micro litres of the content of vial 1 was pipetted into test tubes labelled "reagent blank" and "sample". This was followed by the addition of 100μL of distilled water into the reagent blank tube and the 100μL of serum into the sample test tube. The resulting solutions were well mixed and incubated at 37°C for 30 minutes.

Five hundred micro litres of DNPH was added into each tube, mixed and allowed to stand for exactly 20 minutes at room temperature. Five hundred micro litres of sodium hydroxide was then added to each tube and the absorbance of the test sample read at a wavelength of 546nm against the reagent blank. The concentration of the ALT in the serum was obtained from **Table 3.4**. The reference range for this kit is values up to 12 U/L.

Table 3.4: - Range of absorbance and corresponding ALT activity

Absorbance Range	U/L	Absorbance Range	U/L
0.025 – 0.049	4	0.275 – 0.299	48
0.050 – 0.074	8	0.300 – 0.324	52
0.075 – 0.099	12	0.325 – 0.349	57
0.100 – 0.124	17	0.350 – 0.374	62
0.125 – 0.149	21	0.375 – 0.399	67
0.150 – 0.174	25	0.400 – 0.424	72
0.175 – 0.199	29	0.425 – 0.449	77
0.200 – 0.224	34	0.450 – 0.474	83
0.225 – 0.249	39	0.475 – 0.499	88
0.250 – 0.274	43	0.5	94

Gamma glutamyl transferase (GGT)

In the presence of GGT, glycylglycine reacts with L-gamma-glutamyl-p-nitroanilide to form L-gamma-glutamylglycine and the absorbance is determined kinetically.

L-gamma-glutamyl-p-nitroanilide + glycylglycine

GGT ↓

L-gamma-glutamylglycine + p-nitroalanine

Procedure

The test kit contains two vials; vial 1 contains an already made reagent constituted by "Tris buffer" and glycylglycine and vial 2 contains the substrate containing L-gamma-glutamyl-p-nitroanilide. The test reagent was reconstituted by emptying the entire contents of vial 2 into vial 1 and mixed gently until complete dissolution. One thousand micro litres of the reconstituted reagent was pipetted into a cuvette of 1cm light path and incubated at 37°C. This was followed by the addition of 50µL of the serum sample and the absorbance measured against air at a wavelength of 405nm at 30 seconds and after 1, 2 and 3 minutes and the concentration calculated as follows: -

GGT (IU/L) = change in absorbance per minute X 2121 (molar absorptivity of p-nitroalanine). The reference range for this kit is values up to 50U/L.

Bilirubin

Bilirubin reacts with diazotized sulphanilic acid (DSA) to form a pink azo compound. The absorbance of this compound at 550nm is directly proportional to the bilirubin concentration in the test sample. Total bilirubin concentration is measured when this reaction takes place in the presence of dimethyl sulphoxide (DMSO) and the direct (conjugated) bilirubin is determined when the reaction takes place in the absence of DMSO. Indirect bilirubin = total bilirubin – direct bilirubin.

Procedure

The test kit contains three vials; vial 1 for total bilirubin is constituted by sulphanilic acid, DMSO and hydrochloric acid, vial 2 for direct bilirubin is constituted by sulphanilic acid and hydrochloric acid and vial 3 contains sodium nitrite. The tests for both total and direct bilirubin concentrations were run simultaneously.

To measure total bilirubin, one thousand micro litres of vial 1 was pipetted into test tubes labelled sample blank and assay. Then 50µL of distilled water was added to the sample blank test tube and 50µL of sodium nitrite added to the assay test tube. The contents of both tubes were mixed thoroughly and incubated for 5 minutes at 37°C. After this, 100µL of the test serum was pipetted into each test tube, mixed and allowed to stand for 5 minutes at room temperature. The absorbance of the assay was read against the sample blank at a wavelength of 550nm and the concentration calculated as follows:

µmol/L= absorbance x 195 (a factor given by the manufacturer). The reference range for total bilirubin includes values up to 17.1 µmol/L.

For the measurement of direct bilirubin, the same procedure was used with vial 2 which contained no DMSO. The reference range for direct bilirubin includes values up to 3.4 µmol/L.

Independent quality assessments

Internal quality control of biochemistry tests was ensured by using standard control reagents kits. In addition, three hundred duplicate sera from the Nkoranza Antimalarial Drugs Project were randomly selected and submitted for biochemistry quality assurance testing at NMIMR. At the end of the tests the author detected that more than 50% of bilirubin results came out with total bilirubin as zero while the direct and indirect bilirubin were greater than zero. And so the tests were rerun using the same reagents used by the study laboratory but the results were the same. The author was not satisfied with the results from NMIMR and declined

using them as "gold standard". The samples were sent to the Kintampo Health Research Centre for another assessment. There was substantial delay in getting the results from KHRC. However, the KHCR used different reagents and so again their results were not comparable to those of the study laboratory.

3.6.3.6 Preparation and storage of filter paper blood spots

Filter paper blood spots were prepared for each study pregnant woman on days 0, 3, 7, 14 and 28. Perkin Elmer Filtermat A, (cat 1205-401) filter paper, desiccant sachets, Manila paper and unclotted blood in EDTA tube were use for the preparations.

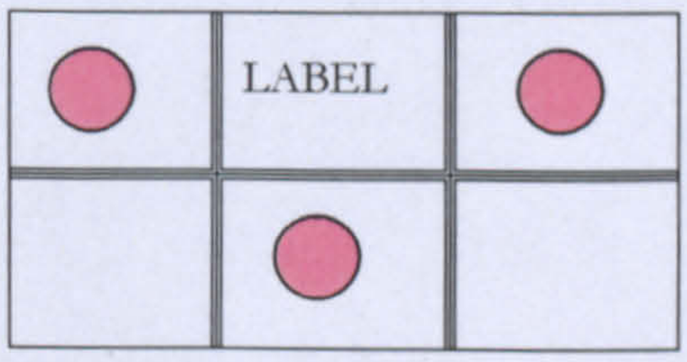
Following each recruitment session or a field visit the filter paper was cut into blocks of six spots and the Manila paper into strips (6ins. X 1.5ins.) in the laboratory according to the number of spots that had to be prepared.

Each filter paper block was stapled onto a Manila paper strip.

Each filter paper block and Manila paper strip was labelled accurately with the pregnant woman's number and the date and day the sample was taken.

Using a 10uL pipette, three 10uL spots of the woman's blood collected into the EDTA tubes were put on each block of filter paper. The drops were allowed to soak into the filter paper until the paper spot was evenly red without spreading to the underlying surface.

Each prepared filter paper blood spots looked as below:



After preparation the batch was dried in a hot air oven at 40°C for 15 minutes.

The dried filters were packed into plastic containers with desiccant in sets according to the day of preparation, sealed and stored at room temperature. Spots from days 0, 3, 7, 14 and 28 were stored separately from each other. The desiccant sachets were replaced every three months throughout the project period until PCR analysis was done at the London School of Hygiene and Tropical Medicine.

3.6.3.7 Urine tests

The enzyme-linked immunosorbent assay (ELISA) (Schwick *et al.* 1998; Mockenhaupt *et al.* 2000) dipstick was used to screen urine samples obtained from study pregnant women for chloroquine and pyrimethamine prior to administration of study drugs to estimate current drug use among the pregnant women in the study area. The dipsticks were obtained directly from the manufacturer, Dr. Teunis Eggelte of the Academic Medical Centre in the Netherlands.

The dipstick test for antimalarial drugs is based on the specific reaction of an antimalarial drug with an antibody raised against it. The dipsticks for CQ and pyrimethamine have 3 bands. The top band shows the reaction between a monoclonal antibody and the enzyme peroxidase and the middle and lower bands contain specific drug antibodies for CQ and pyrimethamine respectively. The dipstick has detection limits in the range of 120nmol/l for CQ and 250nmol/l for SP. The test can detect CQ for at least 3 months and SP at least 3 weeks respectively after they have been taken. The top band acted as an internal control. When a test was negative 3 coloured bands appeared on the dipstick. When a test was positive for CQ, the top and middle bands only were coloured. No tests were positive for pyrimethamine or for both CQ and pyrimethamine. If a test had been positive for pyrimethamine the top and lower bands only would have been coloured. If the test was

positive for both CQ and pyrimethamine the top band alone would be coloured. The assays in this project were performed according to the protocol supplied by the manufacturer.

3.6.3.8 Stool microscopy

At recruitment, the stools of all pregnant women were examined microscopically to identify those with hookworm infestations. Those who were positive were treated with merbendazole 100mg twice daily for 3 days.

3.6.4 Randomisation and study drug allocation

There were four treatment groups: - CQ, SP, AQ or the AQ+SP combination. Treatment allocation was done individually and not in groups. Each drug pack had a numeric drug code, randomly allocated to it by a statistician, which was unknown to the recruiting team. A list of random numbers was computer generated as drug codes and individually linked to identification markers assigned by the manufacturer to each drug pack. These were again randomly ordered in blocks of sixteen, containing equal numbers of the treatment options, into bigger paper envelopes. There were 57 big envelopes, 56 of them had 16 drug packs each individually labelled with the appropriate drug code and the 57th had 4 drug packs. One big envelope was made available at each antenatal clinic section. After giving informed consent, a participant was assigned the lowest available serial identification number. She was then asked by the recruitment team to pick a sealed drug pack from the big envelope and the drug code assigned to the pack was recorded. Picking a drug pack constituted entering the study, and the drug allocation within it was binding on the recruiting team and the woman at this point. Another big envelope was opened only when the contents of the previous one was exhausted. The 57th envelope was the last to be opened. The PI and project staff were blinded to the randomisation process and treatment allocation.

The test drugs were formulated as film-coated oral tablets and had the same appearance in size and colour. They were pre-package in blister packs and strips by the manufacturer.

All the drugs were manufactured by Kinapharma Limited in Ghana and quality assurance tested by the Foods and Drugs Board of Ghana. Their solubility and drug content was confirmed by high pressure liquid chromatography at the London School of Hygiene and Tropical Medicine by Dr. Harpakash Kaur.

3.6.5 Treatment Schedule and Dosage

The test drugs and their dosages and treatment schedules are shown in **Table 3.5.**

Table 3.5: Schedule and dosage of test drugs

TREATMENT ARM	DAY 0	DAY 1	DAY 2
CQ	CQ (600mg) PLACEBO 1	CQ (600mg)	CQ (300mg)
AQ	AQ (600mg) PLACEBO 1	AQ (600mg)	AQ (400mg)
SP	S/P (1500mg/75mg) PLACEBO 2	PLACEBO 2	PLACEBO 2
AQ+SP	AQ (600mg) S/P (1500mg/75mg)	AQ (600mg)	AQ (400mg)

A drug pack contained a silver strip of three tablets and blister pack of a yellow and two pink tablets. A drug pack appeared as below: -

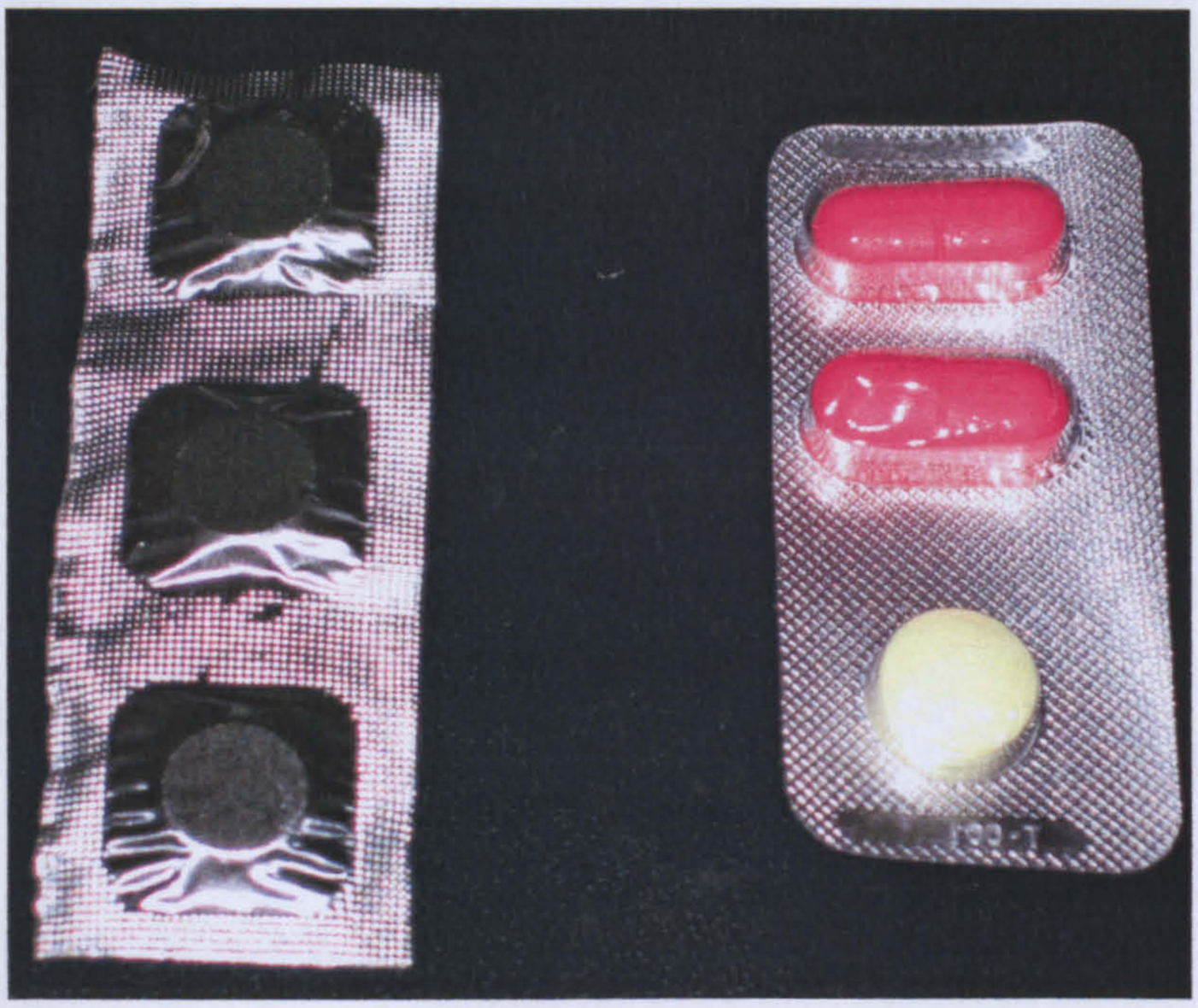


Figure 3.4: Contents of individual drug packs

The silver strip was either an active SP or a placebo and the blister pack of pink and yellow tablets was either CQ or AQ monotherapies in which cases the silver strip was a placebo, or in the case of the AQ+SP combination, both the silver strip and the blister packs were active drugs.

3.6.6 Drug compliance and accountability

In addition to directly observing intake of the first dose of antimalarials at the clinic, field staff inspected the empty drug packages at home to ensure that the women had taken the drugs on their visits.

Figure 3.5: - Direct observation of the first treatment dose

3.7 Follow-up procedures

3.7.1 Follow-up visits.

Field workers visited pregnant women in their homes following the initial supervised drug administration at the antenatal clinic on days 3, 7, 14 and 28. To ensure that all study pregnant women were identified and followed up by field workers, the following procedures were followed.

1. On the day before a follow up visit the data entry team generated a list of pregnant women due to be visited the following day.
2. The list contained details such as address, contact persons and landmarks to facilitate identification of the pregnant women in the communities.
3. The data entry team supplied the field teams with the appropriate forms for each visit. There were separate but similar follow up forms (F-Forms) designed for the purpose and kept by the data entry team.

3.7.1.1 Follow up on days 3 and 7

1. The field workers located and identified the study pregnant woman in the community.
2. They identified themselves to the woman and her husband or family.
3. The field workers asked about the health of the woman and recorded any complaints.
4. They recorded any adverse effects attributable to the ingestion of the test drugs.
5. In an unthreatening manner, they inspected drug packages to ensure that every tablet was accounted for.
6. They recorded the axillary temperature of the study woman and noted any observable external physical changes such as pallor, jaundice or skin rash.
7. They sought permission and obtained a 2-ml sample of venous blood from the woman into a tube containing the anticoagulant ethylene di-amine-tetra-acetic acid

(EDTA) to be used for the preparation of thick films, WBC (total and differential), and for subsequent filter paper blood spots in the laboratory.

8. They recorded all follow up information on follow up forms (F Forms).
9. At the end of each encounter, they checked that all sections of the form were fully completed.
10. The field workers then gave notice for the next visit on days 7, 14 and 28 and bid the woman and her family good bye.
11. Back in the laboratory at St. Theresa's Hospital, the field worker prepared the filter paper spots and prepared thin and thick blood smears for each woman seen in the field.
12. They accurately labelled all slides and filter papers and completed a separate laboratory form (L-Form) for each slide and each filter paper sample.
13. The field supervisor ensured that all samples expected from the field had been collected.
14. He inspected all samples to ensure that they were properly labelled and all forms fully completed.
15. After verification the field supervisor submitted the samples to the laboratory technician for examination.
16. The laboratory technician, after examining the slides, filled in the results on the L-Form and returned them to the field supervisor.
17. The field supervisor and the field teams then met the PI with the laboratory results and records from the field.
18. The PI checked that no information was carelessly left out by ensuring that each woman's F Form 3 was fully completed.
19. The PI then initialled the forms and submitted them to the data entry team.

3.7.1.2 Follow up on days 14 and 28

The above follow up routine was repeated on each of the subsequent follow-up visits for each pregnant woman. However, on days 14 and 28, 5-ml samples of venous blood were obtained. One ml was added to an EDTA tube to be used for preparing thin and thick films, haemoglobin concentration assessment, white blood cell counts (total and differential), and for the preparation of filter paper blood spots. The rest was allowed to clot to separate sera from the cells and centrifuged to give approximately 2mls of serum. This was divided into two portions, one was kept as a duplicate sample and the other was used for the liver function tests. Sera were kept frozen at –20°C until analysed.

3.7.2 Subsequent antenatal visits

All study women were seen at the antenatal clinic monthly or fortnightly for those with 32 weeks and above gestation. At these visits, the women were screened actively for peripheral parasitaemia using an OptiMAL® dipstick test. If a woman who had already enrolled had a positive antigen test confirmed by microscopy, she received another course of the treatment to which she had been initially assigned. However, analyses were restricted only to the first treatment.

3.7.3 Delivery and postpartum follow-up

Midwives were charged with recording all birth weights and placenta weights, and noted any stillbirths and perinatal deaths. They prepared thick blood films from cord blood and from the maternal surface of the placenta; these were examined for malaria parasites and pigment at the laboratory after Giemsa staining. A clinician confirmed any record of congenital deformity. All women and babies were followed for six weeks during which their self-reported health status was obtained and reports of any neonatal adverse events such as

deaths or morbidity ascertained. The women were also advised to continue the nationally approved weekly 300mg of chloroquine over 6 weeks postnatal period.

3.8 Iron and folic acid supplementation

All study pregnant women received monthly pre-packed supply of prophylactic iron (as ferrous sulphate 200mg containing 65mg iron) and folic acid (4mg) tablets. One tablet each of these was to be taken daily.

3.9 Treatment of Maternal Anaemia

This study defined *anaemia* according to the WHO criteria as a haemoglobin concentration (Hb) below 11g/dl and *severe anaemia* as an Hb below 7g/dl. Apart from the routine administration of iron (200mg ferrous sulphate equivalent to 60mg elemental iron) and folate (400mcg) supplementation during pregnancy, anaemia in any study pregnant women was actively managed depending on the level of Hb, presence of symptoms of cardiovascular decompensation and gestational age.

The following regimen was used to actively treat antenatal anaemia (Kwame-Aryee 1998):

Hb of 5 – 7g/dl at less than 36 weeks gestation.

Asymptomatic

Oral ferrous sulphate 200mg three times daily on an OPD basis.

Symptomatic

Blood transfusion followed by oral ferrous sulphate.

Identification of any causes of the anaemia and provision of appropriate treatment for the underlying condition.

After delivery or after the Hb had returned to normal levels oral iron was continued for 3 to 6 months in order to replenish the iron stores.

3.10 Escape Medication

Oral or parenteral[1] quinine sulphate given as 10mg/kg every 8 hours for 7 days was used as an escape medication if: -

i. There was deterioration in a woman's clinical condition after initiating treatment[2].
ii. The level of parasitaemia on day 3 or 7 was higher than that of day 0.
iii. Any level of parasitaemia was found on days 14 and 28 after initiating treatment.
iv. A woman with malaria parasitaemia refused to continue with study medication because of adverse effects.

3.11 Assessment of adverse drug events

An adverse event was defined as any untoward medical or obstetric occurrence (including physical and laboratory occurrences) in a pregnant woman, which may or may not have been related to the test drugs and which developed or increased in severity within seven days of initiating test treatment. Women were monitored for these signs and symptoms during post-treatment home visits or if they self reported an event. The PI or study clinician reviewed the information from the field and made judgements about the nature (severity and seriousness) of the event and actions to be taken according to the following predefined guidelines.

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[1] If oral medication was not possible but this did not happen.
[2] This situation did not arise.

Severity of a reported adverse event

1. An event was judged to be mild if it did not interfere with the woman's routine chores and required no specific intervention.
2. An event was judged to be moderate if it interfered with the woman's routine chores or compelled the woman to suspend the test treatment or if she required any specific intervention.
3. An event that required a supervised intervention such as admission to hospital was judged to be severe.

Seriousness of adverse events

An adverse drug event was judged to be serious if the ingestion of a test drug resulted in death of the subject or in a life-threatening situation and not serious if it was not life threatening and could not cause any damage.

In the context of this study the occurrence of the following medical and/or obstetric outcomes known to be related to the test drugs were classified as serious and monitored within 7 days following ingestion of a test drug.

1. Steven Johnson's syndrome: this is a toxic cutaneous reaction to drugs causing painful blistering and sloughing of the skin and mucous membranes including those of the mouth and eye.
2. Hepatitis, as indicated by jaundice and abnormal levels of serum bilirubin and liver transaminases,
3. Leucopoenia as indicated by abnormally low levels of white blood cells (below 2 x 10^9 /litre).
4. Miscarriage.
5. Neonatal jaundice as indicated by yellowish discoloration of the baby with 48 hours of delivery

Attributing causality

The relation of the AE to the ingestion of the drug was assessed as: -

Unlikely

The event was clearly related to other factors such as concomitant therapy or a woman's clinical state, and was unlikely to have been caused by the study drug.

Possible

The event followed a reasonable temporal sequence from the time of ingesting study drug and/or followed a known response pattern to the study drug, but could have been produced by other factors such as the underlying illness or concomitant therapy.

Probable

The event followed a reasonable temporal sequence from the time of ingesting study drug and/or followed a known response pattern to the study drug, and was unlikely to have been produced by other factors such as the underlying illness or concomitant therapy.

Most probable

The event followed a reasonable temporal sequence from the time of ingesting study drug and/or followed a known response pattern to the study drug, could not have been produced by other factors such as the underlying illness or concomitant therapy, and either occurred immediately following the study drug ingestion or improved on stopping the drug.

Actions taken in response to a reported adverse event

The detection of an AE could lead to the following actions: -

None

No action taken and study drug given as normal.

Study drug discontinued

Study woman stopped taking the study drug and was given escape medication or other antimalarial drugs to complete full treatment for malaria.

Other action taken

This referred only to concomitant therapy (not anti-malarial) or other actions needed to manage the adverse event.

3.12 Withdrawal from study

Study women were withdrawn from the study for the following reasons:

- Self-administration of additional antimalarial drugs during follow-up.
- Emergence of any concomitant febrile illness that might interfere with measurement of outcomes.
- Withdrawal of informed consent.
- Development of severe malaria or danger signs on day of treatment or after leaving the clinic.
- Termination of pregnancy at term or prematurely.

3.13 Data handling and analysis

3.13.1 Data collection and entry

Information obtained from women was recorded on case record forms for each woman enrolled in the study both at the antenatal clinic and during field visits.

Two data entry clerks entered data from record forms into a Microsoft Access 2000 computerized database at St. Theresa's Hospital simultaneously and independently each time throughout the study period. The "Compare Programme" a Microsoft Access programme (B. Beard et al.) for comparing double entered data was used weekly to verify that records in

both databases were the same. Stata (version 8) software was used to clean and validate entered data and for performing the main analysis. Prior to analysis the related data files - baseline, follow up, laboratory and post delivery data were processed and merged to produce the main file for analysis.

3.13.2 Data analysis

The principal analyses of primary and secondary outcomes employed the "intention-to-treat" approach to include all randomised participants in their randomly assigned treatment group. Treatment group assignment was not altered based on the participant's adherence to the assigned treatment regimen during the study. All statistical tests performed were two-sided.

Baseline data

The background characteristics and baseline measures including age, parity, gestation, gravidity, prior chloroquine use, presenting symptoms, bed net availability and usage, spleen and liver enlargement, level of education, occupation, residence, ethnicity, religion, and haematological and biochemistry data were summarised using descriptive statistics.

Binary variables were presented with frequencies and percentages, distributions for continuous variables will be summarised with means and standard deviations (SD) or medians and interquartile range (IQR).

Post treatment analyses

Mainly, the post treatment analyses involved the comparison of AQ, SP and AQ + SP to CQ with respect to the primary and secondary end points.

The primary analysis involved comparisons of proportions of parasitological failure for each treatment compared to CQ at fixed time points of follow up. A failure was defined as a

Plasmodium falciparum density on days 3 and 7 follow up which was greater than that observed at baseline or any level of parasitaemia on days 14 and 28 following treatment. Bar charts of parasitological failures were also displayed to compare the effect of each test treatment versus CQ by days 14 and 28 following treatment. Odds ratios, 95% CI's and corresponding p-values were estimated and tabulated for 14 and 28 days after treatment.

Secondary analyses

Treatment effect on haemoglobin, liver enzymes, bilirubin and white blood cells was assessed by measuring the absolute change from baseline levels over time using paired t-test, analysis of variance (ANOVA) and multiple regression analyses. For haemoglobin, the change at delivery and postpartum was also assessed.

Haemoglobin, AST, ALT, GGT, bilirubin and white blood cells levels were also categorised based on reference ranges. The number of values in each category was tabulated with the corresponding percentage of the total number of values available according to treatment group. The overall estimates were presented unless there were any significant differences between subgroups in which case estimates were presented for each stratum.

The experience of an adverse effect by a woman and the incidence of each individual adverse effect reported by women during post treatment follow up on days 3, 7, 14 and 28 were tabulated according to treatment group. Estimates of treatment effect using odds ratios and their 95% confidence intervals and corresponding P-values were obtained for each event by treatment group AQ, SP and AQ+SP versus CQ. Line and bar graphs were used to display patterns of adverse events both within and across treatment groups over time.

It was anticipated that the signs and symptoms relating to the underlying parasitaemia may confound these estimations and so estimates were adjusted for baseline variables. In addition, clinical information obtained from the parallel screening and follow up of untreated non-

parasitaemic pregnant women during the study were compared with those of enrolled parasitaemic pregnant women.

Pregnancy outcomes of women in the study were compared according to treatment group using descriptive statistics. Also the overall incidence of adverse pregnancy outcomes in the study group was compared to local rates for abortion, stillbirth, congenital abnormality, and mean gestation at delivery obtained from St Theresa's Hospital's records.

3.13.3 Additional Data

The overall parasitological failure rate of the study drugs in the study population appeared to be low during an interim review of the trial progress in February 2004. Even if all the failure at the time was from the chloroquine group it was thought not to be consistent with available knowledge. This could be due to the parasitological effect of the test drugs being different in pregnant women from what is seen in children (from whom most of the available data was obtained); or the parasite resistance in the study area being low compared to other parts of the country.

And so an open-label randomised controlled trial was commissioned in which of 203 children with peripheral parasitaemia were assigned SP or CQ to assess the parasitological failure by days 14 and 28 after treatment. Similar methods used for the primary analysis of the main study were used to estimate the efficacies of CQ and SP malaria infection in children in the study area. The drugs were from the source as in the main study. The CQ came as tablets colour coded and pre-packed; the first dose was given at the OPD under supervision. The SP came as syrup and was administered as a single dose under observation at the OPD before the children left the hospital. The results are shown in **Appendix 16.**

A control group of 220 pregnant women with negative OptiMAL® antigen tests during screening were also randomly selected, assessed clinically and followed up over 28 days using the same case records forms as for those in the study. The aim was to have a baseline level of clinical complaints among pregnant women in the study area.

3.14 Ethical Approval

The ethics committees of the Health Research Unit of the Ministry of Health of Ghana and the London School of Hygiene and Tropical Medicine gave approval for the study.

3.15 Protocol Amendments

No protocol amendments were required.

CHAPTER FOUR

SCREENING AND ENROLMENT

CHAPTER 4 SCREENING AND ENROLMENT

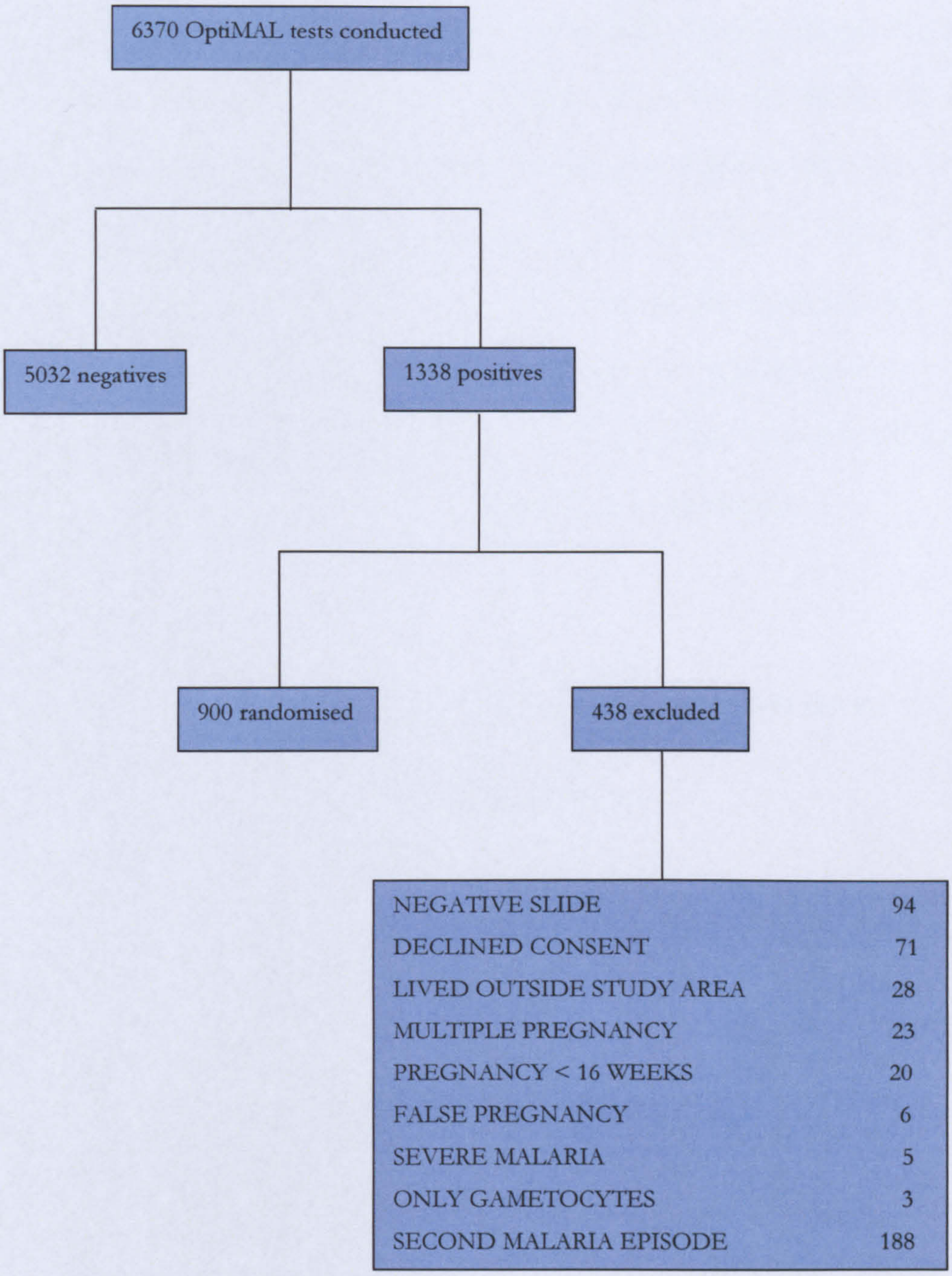
4.1 Introduction

The first section of this chapter begins with a description of the screening processes that led to randomisation of eligible pregnant women into the study. This is followed by a description of the demographic and social characteristics of the study women, their clinical and obstetric assessments and baseline parasitological and haematological indices.

4.2 Screening and enrolment

A flow diagram of the screening process that led to randomisation and enrolment of pregnant women into the study is shown in **Figure 4.1.** A total of 6370 OptiMAL® tests were performed on 4500 pregnant women who attended a routine antenatal clinic; 1338 (21%) were positive and 5032 were negative. Ninety-four of the 1281 positives assessed microscopically, were negative. Fifty-seven of the positive slides were not read microscopically because the women declined consent before further samples could be taken or the screening process completed. Five hundred and fifty-four of the 598 negative dipstick tests assessed microscopically were confirmed negative and 44 (7.3%) were positive. Nine hundred pregnant women who satisfied the study inclusion criteria were enrolled into the study and 438 excluded for the reasons indicated in **Figure 4.1.** One hundred and eighty-eight who were enrolled earlier and successfully completed the initial 28-day follow up tested positive during subsequent screening sessions. They did not re-enter the study.

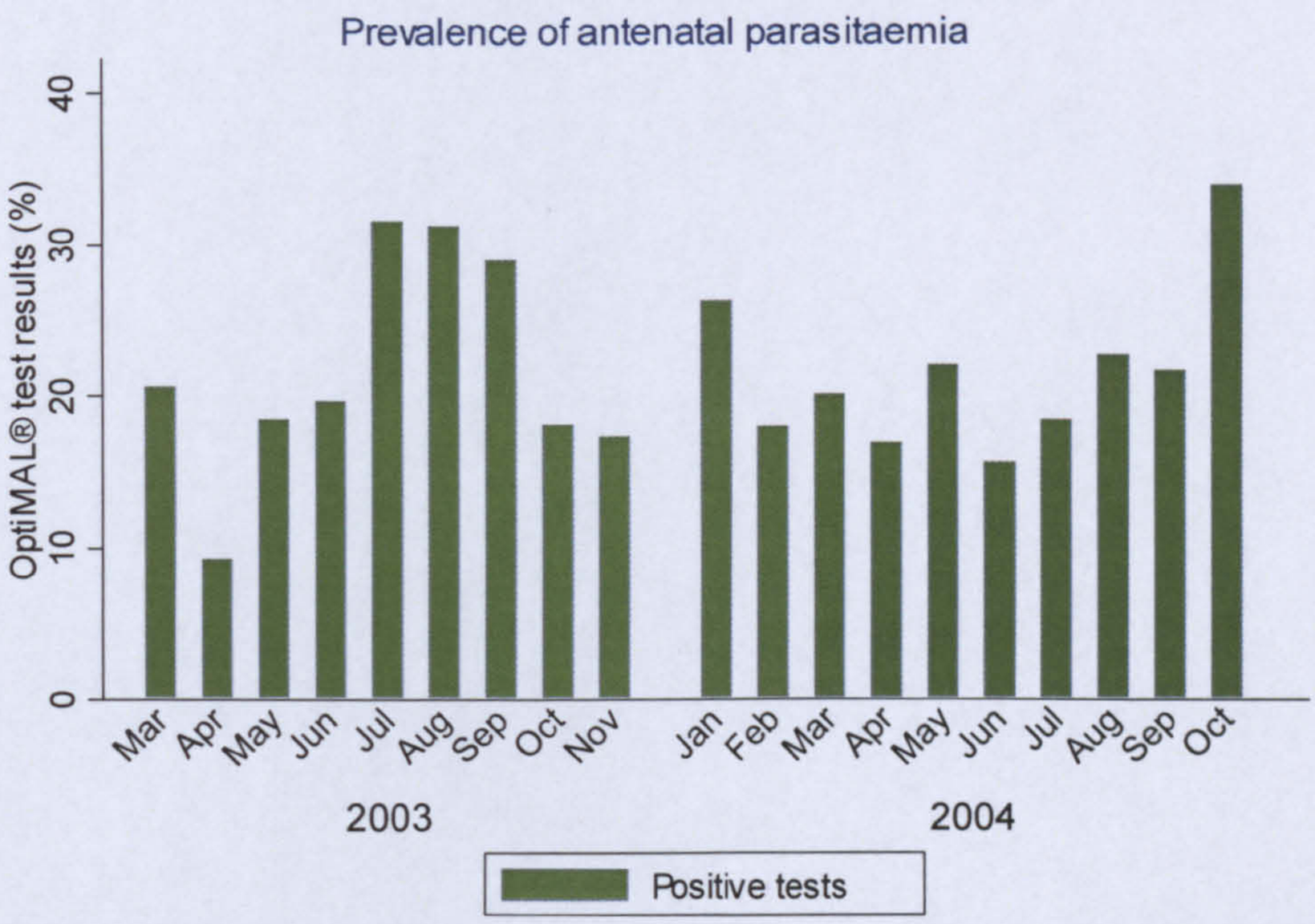
Figure 4.1: Flow diagram of the screening process that led to enrolment of pregnant women into the study.



4.2.1 *Prevalence of peripheral parasitaemia in the study population*

Screening of pregnant women for enrolment into the study began on March 18, 2003 and continued until October 2004 as shown in **Figure 4.2**. Over this period, the average prevalence of peripheral parasitaemia among antenatal clinic attenders was about 22% with monthly variations ranging from 9% to 32% in 2003 and from 16% to 34% in 2004. The prevalence peaked in June, July and August in 2003 during the rainy season but did not show any clear pattern relating to the dry and wet seasons of the study area during the year of 2004. The main parasite species in the study area is *Plasmodium falciparum*. No mixed infections were detected in the study population either by OptiMAL® test or microscopy.

Figure 4.2: - Detection of antenatal parasitaemia by OptiMAL® test over study period.



Factors associated with a positive dipstick test result at screening are shown in **Table 4.1.** Low parity, gravidity and age were associated significantly with positive dipstick tests ($p<0.001$).

Table 4.1: - Univariate analysis of factors associated with a positive OptiMAL® test.

	OptiMAL® test result						
	Negative		Positive		Unadjusted odds ratio		
	n	(%)	n	(%)		(95% CI)	LR test
Parity							
2 or above	2,609	(51.9)	323	(24.1)	1.0		**p<0.001**
1	1,163	(23.1)	281	(21)	2.0	(1.7 - 2.3)	
0	1,260	(25)	734	(54.9)	4.7	(4.1 - 5.5)	
Gravidity							
3 or above	2,743	(54.5)	353	(26.4)	1.0		**p<0.001**
2	1,167	(23.2)	324	(24.2)	2.2	(1.8 - 2.5)	
1	1,122	(22.3)	661	(49.4)	4.6	(3.9 - 5.3)	
Gestation							
3rd trimester	2,833	(56.3)	702	(52.5)	1.0		**p=0.03**
2nd trimester	2,176	(43.3)	632	(47.2)	1.2	(1.0 - 1.3)	
1st trimester	20	(0.4)	4	(0.3)	0.8	(0.3 - 2.3)	
Mean (SD)	26.6	(6.5)	25.9	(6.4)			
Median (IQR)	28	(12)	26	(12)			
Age							
30+	1,643	(32.7)	187	(14)	1.0		**p<0.001**
25-29	1,384	(27.5)	261	(19.5)	1.7	(1.4 - 2.0)	
20-24	1,441	(28.6)	496	(37.1)	3.0	(2.5 - 3.6)	
<20	563	(11.2)	394	(29.4)	6.1	(5.0 - 7.5)	
Mean (SD)	26.8	(6.3)	23	(5.6)			
Median (IQR)	26	(8)	22	(7)			

Nulliparous women (OR=4.7; 95% CI, 4.1 to 5.5) and primiparous women (OR=2.0; 95% CI, 1.6 to 2.3) respectively were more likely to have positive dipstick tests compared to multiparous women in the study population. Similarly primigravidaes (OR=4.6; 95% CI, 4.0 to 5.3) and secundigravidaes (OR=2.2; 95% CI, 1.8 to 2.5) respectively were more likely than multigravidaes to have positive dipstick results. Pregnant women less than 30 years of age were more likely than those 30 years or above to have positive dipstick results as shown in

Table 4.1. Gestational age at screening was associated significantly with a positive dipstick test result (p=0.03); women in their second trimester (OR=1.2; 95% CI, 1.0 to 1.3) were more likely to have a positive test result than those screened in their third trimester.

4.2.2 Performance of the OptiMAL® antigen test

The results of parasite detection by the OptiMAL® dipstick and microscopy are shown in **Tables 4.2 and 4.3**. Using microscopy as the gold standard, the OptiMAL® dipstick was found to be highly sensitive (100%) and specific (93.3%) for the diagnosis of *P. falciparum* infection in pregnancy with a negative predictive value of 100% for parasite densities above 50/μL of whole blood. However, the sensitivity of the test was reduced to 57.1% with a specificity of 93.3% for parasite density below 50/μL. When the dipstick was compared with microscopy results labelled as negative or positive the dipstick had a sensitivity of 96.4% and a specificity of 85.5%.

Table 4.2: - The performance of the OptiMAL® test versus peripheral microscopy in the diagnosis of malaria infection in pregnancy.

	MICROSCOPY											
	Parasite level <50/ul			Parasite level 50 - 100/ul			Parasite level >100/ul			Overall assessment		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
OptiMAL® Test												
Positive	24	9	33	68	9	77	822	9	831	1,187	94	1,281
Negative	18	125	143	0	125	125	0	125	125	44	554	598
Total	42	134	176	68	134	202	822	134	956	1,231	648	1,879

Table 4.3: - Performance of the OptiMAL® dipstick overall and according to the level of peripheral parasitaemia during pregnancy.

Parasitaemia (parasites/μL of whole blood)	Sensitivity (% [95% CI])	Specificity (% [95% CI])	Positive PV* (% [95% CI])	Negative PV (% [95% CI])	Accuracy	Likelihood ratio		Area Under ROC† curve (95% CI)
						Positive test	Negative test	
<50	57.1(49.8 - 64.5)	93.3 (89.6 - 97)	72.7 (66.6 - 79.3)	87.4 (82.5 - 92.3)	84.7	8.5	0.46	0.75 (0.67 - 0.83)
50 - 100	100	93.3 (89.8 - 96.7)	88.3 (83.9 - 92.7)	100	95.5	14.9	<0.001	0.97 (0.95 - 0.99)
>100	100	93.3 (91.7 - 94.9)	98.9 (98.3 - 99.6)	100	99.1	14.9	<0.001	0.97 (0.95 - 0.99)
Overall Assessment (Positive/Negative)	96.4 (95.6 - 97.3)	85.5 (83.9 - 87.1)	92.7 (91.5 - 93.8)	92.6 (91.5 - 93.8)	92.7	6.6	0.04	0.91 (0.9 – 0.92)

* PV = Predictive value
† ROC = Receiver operating characteristics

4.3 Baseline characteristics

4.3.3 Demographic and social characteristics

The baseline demographic and social characteristics of pregnant women in the four treatment groups are shown in **Table 4.4**. The groups were similar with respect to all the characteristics recorded. In all the groups more than half of the women were below 25 years of age with an overall mean age of 23 years. Seventy percent of the women had received a formal education, mainly up to the junior secondary school level (44%). Fifty-seven percent of them were from the Brong ethnic group and mainly farmers (40%) or traders (29%). Twenty-five percent of the study population households had a bed net but only 14% of the study pregnant women slept under a bed net.

4.3.4 Clinical and obstetric characteristics

The results of the clinical and obstetric assessments of the women according to treatment group are shown in **Table 4.5 and Table 4.6** respectively. There were no statistical significant differences between groups. Fifty-eight percent of the women admitted to not being well in the last 5 days prior to enrolment. Headache, fever, general malaise, dizziness and easy fatigability were their main complaints. However, only 7% of them had an axillary temperature of 37.5°C or above at enrolment; 4.1% and 0.9% had a palpable spleen and liver respectively at enrolment. Thirty-six percent gave a history of a drug reaction in the past, mainly itching due to chloroquine. Primigravidaes and nulliparae formed 52% and 55% respectively of the study population.

Table 4.4: - Demographic and social characteristics of study pregnant women

	CQ (N=225)		AQ (N=225)		SP (N=225)		AQ+SP (N=225)		Total (N=900)	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Age [years]										
<20	66	(29.3)	87	(38.7)	74	(32.9)	59	(26.2)	286	(31.8)
20-24	82	(36.4)	77	(34.2)	82	(36.4)	90	(40)	331	(36.8)
25-29	46	(20.4)	32	(14.2)	38	(16.9)	46	(20.4)	162	(18)
30+	31	(13.8)	29	(12.9)	31	(13.8)	30	(13.3)	121	(13.4)
Mean (SD)	23.1	(5.6)	22.5	(5.9)	22.6	(5.4)	23.1	(5.3)	22.8	(5.6)
Median (IQR)	22	(7)	20	(7)	21	(7)	22	(8)	21	(7)
Educational level reached										
None	66	(29.3)	63	(28)	66	(29.3)	71	(31.6)	266	(29.6)
Primary	44	(19.6)	46	(20.4)	50	(22.2)	46	(20.4)	186	(20.7)
JSS	97	(43.1)	105	(46.7)	95	(42.2)	96	(42.7)	393	(43.7)
SSS	12	(5.3)	11	(4.9)	9	(4)	7	(3.1)	39	(4.3)
Tertiary	6	(2.7)	0	(0)	5	(2.2)	5	(2.2)	16	(1.8)
Religion										
None	13	(5.8)	10	(4.4)	12	(5.3)	14	(6.2)	49	(5.4)
Christian	188	(83.6)	188	(83.6)	192	(85.3)	196	(87.1)	764	(84.9)
Islam	23	(10.2)	24	(10.7)	21	(9.3)	14	(6.2)	82	(9.1)
Traditional African	1	(0.4)	3	(1.3)	0	(0)	1	(0.4)	5	(0.6)
Ethnic Groups										
Akans	7	(3.1)	10	(4.4)	8	(3.6)	9	(4)	34	(3.8)
Brongs	123	(54.7)	133	(59.1)	125	(55.6)	135	(60)	516	(57.3)
Dagabas	38	(16.9)	35	(15.6)	43	(19.1)	33	(14.7)	149	(16.6)
Dagombas	9	(4)	6	(2.7)	1	(0.4)	2	(0.9)	18	(2)
Frafras	46	(20.4)	37	(16.4)	47	(20.9)	42	(18.7)	172	(19.1)
Others	2	(0.9)	4	(1.8)	1	(0.4)	4	(1.8)	11	(1.2)
Occupation										
Farmer	81	(36)	94	(41.8)	104	(46.2)	84	(37.3)	363	(40.3)
Housewife	30	(13.3)	24	(10.7)	33	(14.7)	35	(15.6)	122	(13.6)
Salary Worker	9	(4)	3	(1.3)	2	(0.9)	2	(0.9)	16	(1.8)
Trader	67	(29.8)	71	(31.6)	50	(22.2)	69	(30.7)	257	(28.6)
Other	38	(16.9)	33	(14.7)	36	(16)	35	(15.6)	142	(15.8)
Marital Status										
Married	174	(77.3)	176	(78.2)	178	(79.1)	172	(76.4)	700	(77.8)
Single	51	(22.7)	49	(21.8)	47	(20.9)	53	(23.6)	200	(22.2)
Mean household size (SD)	6	(3)	6	(3)	6	(3)	6	(3)	6	(3)
Households with a bed net	63	(28)	47	(20.9)	53	(23.6)	59	(26.2)	222	(24.7)
Bed net Use	33	(14.7)	30	(13.3)	28	(12.4)	38	(16.9)	129	(14.3)

No statistical differences between the groups p>0.05.

Table 4.5: - Baseline clinical characteristics according to treatment group

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Sick within last 5 days										
No	94	(41.8)	93	(41.3)	97	(43.1)	96	(42.7)	380	(42.2)
Yes	131	(58.2)	132	(58.7)	128	(56.9)	129	(57.3)	520	(57.8)
Fever										
No	133	(59.6)	127	(57.2)	126	(56.5)	116	(52)	502	(56.3)
Yes	90	(40.4)	95	(42.8)	97	(43.5)	107	(48)	389	(43.7)
Headache										
No	120	(53.8)	125	(56.1)	123	(55.2)	109	(49.1)	477	(53.5)
Yes	103	(46.2)	98	(43.9)	100	(44.8)	113	(50.9)	414	(46.5)
Vomiting										
No	193	(86.6)	193	(85.8)	197	(87.6)	196	(88.3)	779	(87)
Yes	30	(13.5)	32	(14.2)	28	(12.4)	26	(11.7)	116	(13)
Malaise										
No	141	(62.9)	157	(69.8)	158	(70.2)	151	(68)	607	(67.7)
Yes	83	(37.1)	68	(30.2)	67	(29.8)	71	(32)	289	(32.3)
Cough										
No	201	(90.1)	194	(86.2)	198	(88)	199	(90.1)	792	(88.6)
Yes	22	(9.9)	31	(13.8)	27	(12)	22	(9.9)	102	(11.4)
Dizziness										
No	175	(78.1)	178	(79.1)	182	(80.9)	170	(76.9)	705	(78.8)
Yes	49	(21.9)	47	(20.9)	43	(19.1)	51	(23.1)	190	(21.2)
Dysuria										
No	207	(92.4)	208	(92.4)	199	(88.4)	208	(93.7)	822	(91.7)
Yes	17	(7.6)	17	(7.6)	26	(11.6)	14	(6.3)	74	(8.3)
Easily Tired										
No	172	(76.8)	175	(77.8)	180	(80)	172	(77.5)	699	(78)
Yes	52	(23.2)	50	(22.2)	45	(20)	50	(22.5)	197	(22)
Chloroquine Allergy										
No	142	(63.1)	147	(65.3)	144	(64)	147	(65.4)	580	(64.4)
Yes	83	(36.9)	78	(34.7)	81	(36)	78	(34.6)	320	(35.6)
Past Drug Reactions										
No	142	(63.1)	145	(64.4)	141	(62.7)	146	(64.9)	574	(63.8)
Yes	83	(36.9)	80	(35.6)	84	(37.3)	79	(35.1)	326	(36.2)
Enlarged Spleen										
No	217	(96.4)	215	(95.6)	215	(95.6)	216	(96)	863	(95.9)
Yes	8	(3.6)	10	(4.4)	10	(4.4)	9	(4)	37	(4.1)
Enlarged Liver										
No	222	(98.7)	222	(98.7)	225	(100)	223	(99.1)	892	(99.1)
Yes	3	(1.3)	3	(1.3)	0	(0)	2	(0.9)	8	(0.9)

Table 4.6: - Baseline obstetric characteristics according to treatment group.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Gravidity										
1	126	(56)	121	(53.8)	114	(50.7)	111	(49.3)	472	(52.4)
2	48	(21.3)	52	(23.1)	52	(23.1)	55	(24.4)	207	(23)
3 & above	51	(22.7)	52	(23.1)	59	(26.2)	59	(26.2)	221	(24.6)
Parity										
0	134	(59.6)	126	(56)	118	(52.4)	116	(51.6)	494	(54.9)
1	67	(29.8)	68	(30.2)	77	(34.2)	80	(35.6)	292	(32.4)
2 & above	24	(10.7)	31	(13.8)	30	(13.3)	29	(12.9)	114	(12.7)
Gestation										
2nd trimester	96	(42.7)	99	(44)	104	(46.2)	95	(42.2)	394	(43.8)
3rd trimester	129	(57.3)	126	(56)	121	(53.8)	130	(57.8)	506	(56.2)
Blood Pressure (mmHg)										
Systolic (Mean (SD))	104	(8.1)	103	(9.8)	104	(10)	105	(10.4)	104	(9.6)
Diastolic (Mean (SD))	63	(5.8)	63	(6.8)	63	(6.8)	63	(7.3)	63	(6.7)
Temperature										
<37.5	203	(90.2)	213	(94.7)	211	(93.8)	214	(95.1)	841	(93.4)
37.5°C or above	22	(9.8)	12	(5.3)	14	(6.2)	11	(4.9)	59	(6.6)
Mean (SD)	36.5	(0.8)	36.5	(0.7)	36.4	(0.7)	36.5	(0.7)	36.5	(0.7)
Height/cm										
Mean (SD)	158.6	(6.6)	157.3	(6.5)	157.0	(6.2)	157.7	(6.9)	157.7	(6.6)
Weight/kg										
Mean (SD)	56.4	(8.7)	55.4	(6.8)	55.3	(7.5)	56.5	(8.3)	55.9	(7.8)

4.3.5 Use of chloroquine prior to enrolment

Fifty-one percent of the study women said that they had taken chloroquine within the eight weeks prior to enrolment either for treatment or for prophylaxis as shown in **Table 4.7.** It was not ascertained, however, the proportions who took chloroquine for treatment or for prophylaxis. In sixty-eight percent of these women, this was confirmed by an enzyme-linked immunosorbent assay (ELISA) dipstick testing of the urine at no dilution and in 58% at 100-fold dilution. At zero dilution there was a 58.9% agreement between history and ELISA dipstick in detecting chloroquine use in the last 8 weeks prior to enrolment (kappa statistic of 5.2 and $p<0.001$). At 100-fold urine dilution there was a 59.8% agreement between history and ELISA dipstick in detecting chloroquine use in the last 8 weeks prior to enrolment (kappa statistic = 5.9, $p<0.001$).

Multigravidaes and multiparous women were about one and half times more likely to have used chloroquine prior to enrolment than primigravidae or primiparae.

Table 4.7: - Assessment of chloroquine intake within 8 weeks prior to enrolment.

	Chloroquine detection in urine by ELISA dipstick							
	At zero dilution				At 100-fold dilution			
History of CQ use	Negative		Positive		Negative		Positive	
	n	(%)	n	(%)	n	(%)	n	(%)
No	216	(49.3)	222	(50.7)	269	(61.4)	169	(38.6)
Yes	149	(32.2)	313	(67.8)	193	(41.8)	269	(58.2)
Total	365	(40.6)	535	(59.4)	462	(48.7)	438	(51.3)

4.3.6 Parasitological and haematological indices

The distributions of baseline parasitological and haematological indices by treatment group are shown in **Table 4.8.** The groups were generally similar. However, there were more women with parasite densities higher than 1000/μl in the CQ group than in the other groups (p=0.08). Parasite densities ranged from 32 to 293120 per micro litre of blood. About twenty percent of the women had haemoglobin levels below 8g/dl and only 11% had normal haemoglobin levels at enrolment. The majority of the women (70%) had haemoglobin levels between 8 and 11g/dl. The mean haemoglobin concentration at enrolment was 9.1g/dl.

Table 4.8: - Baseline parasite density and haemoglobin levels according to treatment arm.

	CQ (N=225)		AQ (N=225)		SP (N=225)		AQ+SP (N=225)		Total (N=900)	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Parasite density/µL										
<1000/ul	108	(48)	128	(56.9)	112	(49.8)	131	(58.2)	479	(53.2)
1000/ul & above	117	(52)	97	(43.1)	113	(50.2)	94	(41.8)	421	(46.8)
Geometric Mean	1166		843		1071		842		970	
Range	32 - 293120		32 - 58800		32 - 85333		48 - 194640		32 - 293120	
Haemoglobin										
< 8g/dl	44	(19.6)	39	(17.3)	45	(20)	49	(21.8)	177	(19.7)
8 - 10.9g/dl	156	(69.3)	165	(73.3)	156	(69.3)	151	(67.1)	628	(69.8)
11g/dl & above	25	(11.1)	21	(9.3)	24	(10.7)	25	(11.1)	95	(10.6)
Mean (SD)	9.1	(1.5)	9.2	(1.4)	9.2	(1.5)	9.1	(1.5)	9.1	(1.5)
Median (IQR)	9.1	(1.9)	9.1	(1.8)	9.2	(2.1)	9.2	(2)	9.1	(1.9)

4.3.7 Factors associated with baseline peripheral parasitaemia

Variables associated with parasitaemia at enrolment are shown in **Table 4.9.** In a univariate analysis, baseline peripheral parasite density was found to be associated significantly with age, parity and gravidity ($p<0.001$).

Table 4.9: - Factors associated with antenatal peripheral parasitaemia in the study population.

	Baseline Peripheral Parasitaemia										
	32 - 999/µl		>=1000/µl		Total	Unadjusted odds ratio (95% CI)		p - value*	Adjusted odds ratio† (95% CI)		p – value*
	n	(%)	n	(%)							
Sick within last 5 days	252	(48.5)	268	(51.5)	520	1.6	(1.2 - 2.1)	0.001	1.6	(1.3 - 2.2)	<0.001
Fever	165	(42.4)	224	(57.6)	389	2.2	(1.6 - 2.8)	<0.001	2.2	(1.7 2.9)	<0.001
Vomiting	43	(37.1)	73	(62.9)	116	2.1	(1.4 - 3.2)	<0.001	2.2	(1.5 - 3.4)	<0.001
General malaise	114	(39.5)	175	(60.5)	289	2.3	(1.7 - 3.0)	<0.001	2.4	(1.8 - 3.2)	<0.001
Headache	189	(45.7)	225	(54.3)	414	1.8	(1.4 - 2.3)	<0.001	2.0	(1.5 - 2.6)	<0.001
Easily tired	82	(41.6)	115	(58.4)	197	1.8	(1.3 - 2.5)	<0.001	1.8	(1.3 - 2.5)	<0.001
Dizziness	91	(47.9)	99	(52.1)	190	1.3	(0.9 - 1.8)	0.1	1.2	(0.9 1.7)	0.3
Gravidity											
3 or above	153	(69.2)	68	(30.8)	221	1.0		<0.001			
2	119	(57.5)	88	(42.5)	207	1.7	(1.1 - 2.5)				
1	207	(43.9)	265	(56.1)	472	2.9	(2.1 - 4)				
Parity											
2 or above	149	(72)	58	(28)	207	1.0		<0.001	1.0		<0.001
1	110	(55.3)	89	(44.7)	199	2.1	(1.4 - 3.1)		10.8	(2.3 - 49.7)	
0	220	(44.5)	274	(55.5)	494	3.2	(2.3 - 4.5)		5.6	(1.2 - 25.2)	
Age [years]											
30+	82	(67.8)	39	(32.2)	121	1.0		<0.001	1.0		0.7
25-29	101	(62.4)	61	(37.6)	162	1.3	(0.8 - 2.1)		1.0	(0.5 - 1.7)	
20-24	159	(48)	172	(52)	331	2.3	(1.5 - 3.5)		1.2	(0.7 - 2.2)	
<20	137	(47.9)	149	(52.1)	286	2.3	(1.5 - 3.6)		1.1	(0.6 - 2.2)	
Haemoglobin (g/dl)											
11 or above	59	(62.1)	36	(37.9)	95	1.0		0.07	1.0		0.5
8 - 10.9	336	(53.5)	292	(46.5)	628	1.4	(0.9 - 2.2)		1.2	(0.8 - 2)	
<8	84	(47.5)	93	(52.5)	177	1.8	(1.1 - 3)		1.1	(0.7 - 2)	
Total Bilirubin (µmol/L)											
Up to 17.1	393	(55.1)	320	(44.9)	713	1.0		0.003	1.0		0.005
Above 17.1	66	(41.8)	92	(58.2)	158	1.7	(1.2 - 2.5)		1.7	(1.2 - 2.5)	

* P-values for likelihood ratio test

† Odd ratios adjusted for baseline haemoglobin, parity and age. Gravidity not included in final model.

Compared to multiparous women, nulliparous (OR=3.2 95% CI, 2.3 to 4.5) and primiparous women (OR=2.1; 95% CI, 1.4 to 3.1) were more likely to have parasite densities of 1000/μL or above in the study. Similarly primigravidaes (OR=2.9; 95% CI, 2.1 to 4.0) and secundigravidaes (OR=1.7; 95% CI, 1.1 to 2.5) were more likely than multigravidaes to have higher parasite densities. In multivariate analysis, only parity (being nulliparae or primiparae) remained associated significantly with high baseline parasite density ($p<0.001$). The effects of age and gravidity were confounded by parity. Parity and gravidity are highly correlated with a correlation coefficient of 0.97; $p<0.001$, and when both were included in the same model the effect of parity accounted for both variables. Thus, gravidity was dropped due to the co-linear effect.

Pregnant women with higher parasite densities were more likely than those with densities below 1000/uL to have total bilirubin concentrations above normal (OR=1.7; 95% CI, 1.2 to 2.5). Also, solicited history of ill health, fever, headache, vomiting, easy fatigability and general malaise were associated significantly with higher parasite densities even after adjusting for parity, baseline haemoglobin and age.

The proportions of clinical histories in a control group of pregnant women who did not have peripheral parasitaemia detected are compared with those in the study pregnant women in **Table 4.10**. The proportions of ill health including fever, headache, vomiting, general malaise, tiredness and dizziness were significantly lower in pregnant women without parasitaemia than in those with parasitaemia.

Table 4.10: - Comparisons of proportions of clinical symptoms presented by pregnant women with or without *P. falciparum* parasitaemia.

Total number of women	Parasitaemic [N=900]		Non-parasitaemic [N=223]		p - value††
	n	(%)	n	(%)	
Sick within last 5 days	520	(57.8)	71	(31.8)	<0.001
Elicited signs & symptoms					
Fever	389	(43.7)	14	(6.3)	<0.001
Headache	414	(46.5)	31	(13.9)	<0.001
Vomiting	116	(13)	2	(0.9)	<0.001
Malaise	289	(32.3)	17	(7.6)	<0.001
Dizziness	190	(21.2)	7	(3.1)	<0.001
Easily Tired	197	(22)	6	(2.7)	<0.001
Prior CQ use	462	(51.3)	154	(69.1)	<0.001
Past Drug Reactions	326	(36.2)	93	(41.7)	0.1
Chloroquine Allergy	320	(35.6)	94	(42.1)	0.1
Temperature					
37.5°C or above	59	(6.6)	3	(1.4)	
Mean (SD)	36.5	(0.7)	35.5	(2.6)	
Enlarged Spleen	37	(4.1)	3	(1.4)	0.04
Enlarged Liver	8	(0.9)	1	(0.5)	<0.001

†† Fisher's exact p-value

4.3.8 Factors associated with baseline haemoglobin concentrations

Factors associated with baseline haemoglobin concentration are shown in **Table 4.11**. Anaemia at enrolment was associated significantly with younger mothers, nulliparae and primigravidaes ($p<0.05$). Increased serum bilirubin was also associated significantly with anaemia at enrolment ($p=0.05$). Baseline parasite density only had a borderline association ($p=0.07$) with anaemia at enrolment. When adjustments were made for parity and age only age and bilirubin remained associated with anaemia at enrolment.

Table 4.11: - Factors associated with baseline haemoglobin concentration.

	Hb >=11g/dl		Hb = 5 to 10.9g/dl		Unadjusted odds ratio		LR test	Adjusted odds ratio§		LR test
	n	(%)	n	(%)		(95% CI)			(95% CI)	
Parity										
2 or above	34	(16.4)	173	(83.6)			**p=0.01**			**p=0.9**
1	19	(9.6)	180	(90.4)	1.9	(1.0 - 3.4)		1.1	(0.6 - 2.3)	
0	42	(8.5)	452	(91.5)	2.1	(1.3 - 3.4)		1.1	(0.5 - 2.1)	
Gravidity										
3 or above	35	(15.8)	186	(84.2)			**p=0.01**			**p=0.9**
2	22	(10.6)	185	(89.4)	1.6	(0.9 - 2.8)		0.9	(0.5 - 1.9)	
1	38	(8.1)	434	(91)	2.1	(1.3 - 3.5)		1.1	(0.5 - 2.2)	
Age [years]										
30+	23	(19)	98	(81)			**p<0.001**			**p=0.04**
25-29	25	(15.4)	137	(84.6)	1.3	(0.7 - 2.4)		1.2	(0.6 - 2.4)	
20-24	25	(7.6)	306	(92.4)	2.9	(1.6 - 5.3)		2.7	(1.3 - 5.9)	
<20	22	(7.7)	264	(92.3)	2.8	(1.5 - 5.3)		2.7	(1.1 - 6.3)	
Total bilirubin (µmol/L)										
Up to 17.1	65	(9.1)	648	(90.9)			**p=0.05**			**p=0.05**
Above 17.1	23	(14.6)	135	(85.4)	1.7	(1.0 - 2.8)		1.8	(1.1 - 3.0)	

§ Odds ratios adjusted for parity and age

CHAPTER FIVE

TREATMENT OUTCOMES

CHAPTER 5 TREATMENT OUTCOMES

5.1 Introduction

This chapter presents the parasitological and haematological findings during the initial 28 days of follow up after the start of treatment. The proportions of parasitological failure in the AQ, SP and AQ+SP groups are compared to failures in the CQ group at days 14 and 28 of follow up. Mean changes in haemoglobin over enrolment Hb levels at days 14 and 28 are compared within and between treatment groups. The chapter also describes the relationships between baseline prognostic factors and the parasitological and haematological outcomes of treatment.

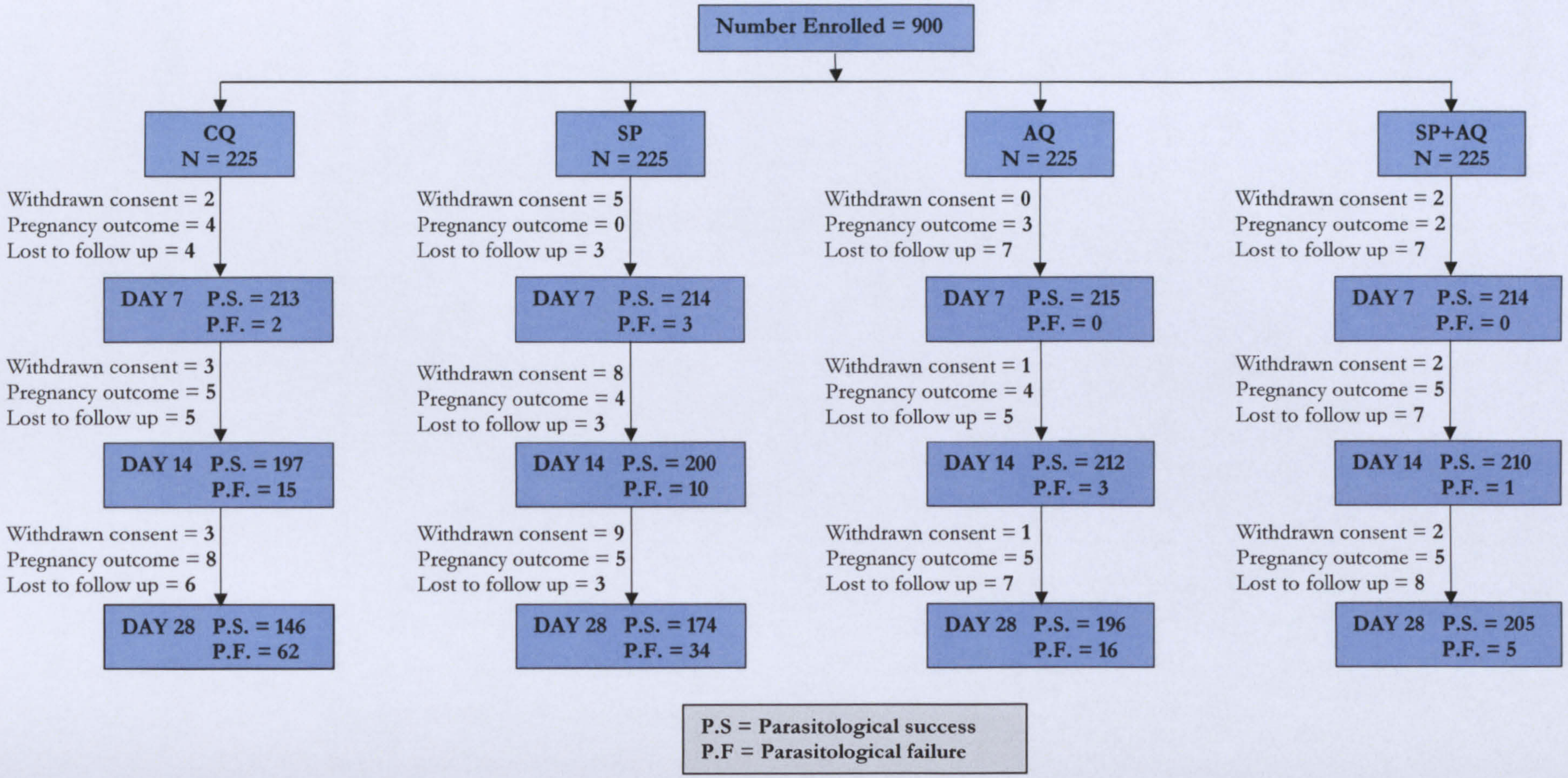
5.2 Participants' flow during 28-day follow up period

The flow of participants from randomisation through the initial 28-day follow-up period is shown in **Figure 5.1**. Nine hundred women were individually randomised and assigned blindly to CQ, AQ, SP or the AQ+SP combination. Thus, there were 225 in each treatment arm. The last woman was enrolled on 24 September 2004. After enrolment, each woman was followed up on scheduled follow up days. A woman was only excluded from subsequent follow ups if she withdrew her consent on a previous follow up visit. During the day 28 follow up period, a woman who was lost to follow up or had experienced a pregnancy outcome was not excluded from subsequent follow ups. However, for women who had experienced a pregnancy outcome during this period, their parasitological and haematological outcomes were not included in the day 14 and day 28 analyses. They were analysed as part of pregnancy outcomes reported in chapter seven.

At day 3 follow up, three women had parasitological failure, five withdrew consent, 22 were lost to follow-up, three of whom were not found on subsequent follow ups; and five had a pregnancy outcome. At day 7 follow up, four withdrew consent, two had parasitological failure, 21 were lost to follow up, four of whom were not found on subsequent follow ups; and four had a pregnancy outcome. At day 14 follow up, five withdrew consent, 24 had parasitological failure, 20 were lost to follow up, two of whom were not found on subsequent follow ups; and nine had a pregnancy outcome. At day 28 follow up, one withdrew consent, 15 were lost to follow up, 88 had parasitological failure, five had a pregnancy outcome and 721 of the women did not have parasitaemia. In all, there were 24 losses to follow-up, 23 pregnancy outcomes, 15 withdrawals of consent, 117 parasitological failures and 721 treatment successes by the end of the initial 28-day follow-up period.

One hundred and eighty-eight women repeated their treatments after the initial 28-day follow-up period when malaria parasites were detected during screening at subsequent antenatal visits. The outcome of pregnancy in this subset of women is analysed separately.

Figure 5.1:- Diagram showing the flow of study pregnant women through the initial 28-day follow-up period.



5.3 Parasitological outcomes

The cumulative incidence of parasitological failure by days 14 and 28 after treatment uncorrected and corrected by msp-2 genotyping are shown in **Tables 5.1.**

Table 5.1: - Parasitological failure by days 14 and 28 after the start of treatment

	Uncorrected parasitological response					Corrected parasitological response				
	Success		Failure		Total	Success		Failure		Total
	n	(%)	n	(%)	N	n	(%)	n	(%)	N
DAY 14										
CQ	197	(92.9)	15	(7.1)	**212**	199	(96.6)	7	(3.4)	**206**
SP	200	(95.2)	10	(4.8)	**210**	202	(97.6)	5	(2.4)	**207**
AQ	212	(98.6)	3	(1.4)	**215**	213	(99.5)	1	(0.5)	**214**
AQ+SP	210	(99.5)	1	(0.5)	**211**	210	(100)	0	(0)	**210**
Total	819	(96.6)	29	(3.4)	**848**	824	(98.4)	13	(1.6)	**837**
DAY 28										
CQ	146	(70.2)	62	(29.8)	**208**	157	(84)	30	(16)	**187**
SP	174	(83.6)	34	(16.4)	**208**	178	(89)	22	(11)	**200**
AQ	196	(92.5)	16	(7.5)	**212**	200	(97.1)	6	(2.9)	**206**
AQ+SP	205	(97.6)	5	(2.4)	**210**	205	(99)	2	(1.0)	**207**
Total	721	(86)	117	(14)	**838**	740	(92.5)	60	(7.5)	**800**

The primary analyses compared outcomes in each treatment group to the outcome in the CQ group. As shown in **Table 5.1,** the cumulative incidence of asexual *P. falciparum* parasitaemia were 0.5%, 1.4%, 4.8% and 7.1% respectively for the AQ+SP combination, AQ, SP and CQ groups by day 14. By day 28 the cumulative incidence of parasitaemia was 2.4%, 7.5%, 16.4% and 29.8% respectively for the AQ+SP, AQ, SP and CQ groups. After correction by msp-2 genotyping, 0%, 0.5%, 2.4% and 3.4% were classified as true failures for the AQ+SP, AQ, SP and CQ groups respectively on day 14. At day 28, 1.0%, 2.9%, 11% and 16% for the AQ+SP, AQ, SP and CQ groups respectively were classified as true failures. A total 38 samples were classified as undetermined because the PCR genotyping failed. The prevalence of parasitological failure by days 14 and 28 according to treatment groups are shown in **Figures 5.2 and 5.2** respectively.

Figure 5.2: - Uncorrected parasitological failure by days 14 and 28 according to treatment group.

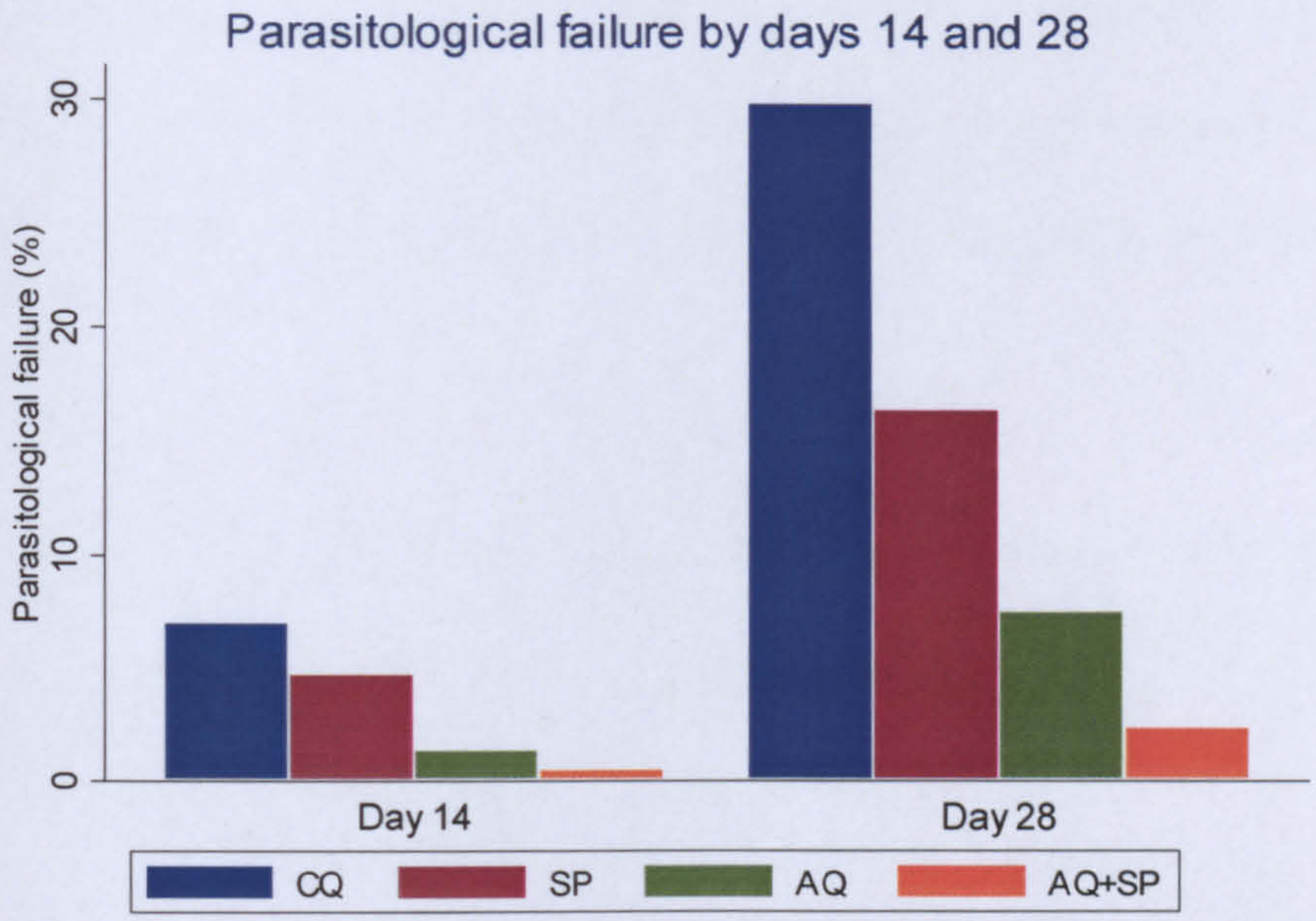
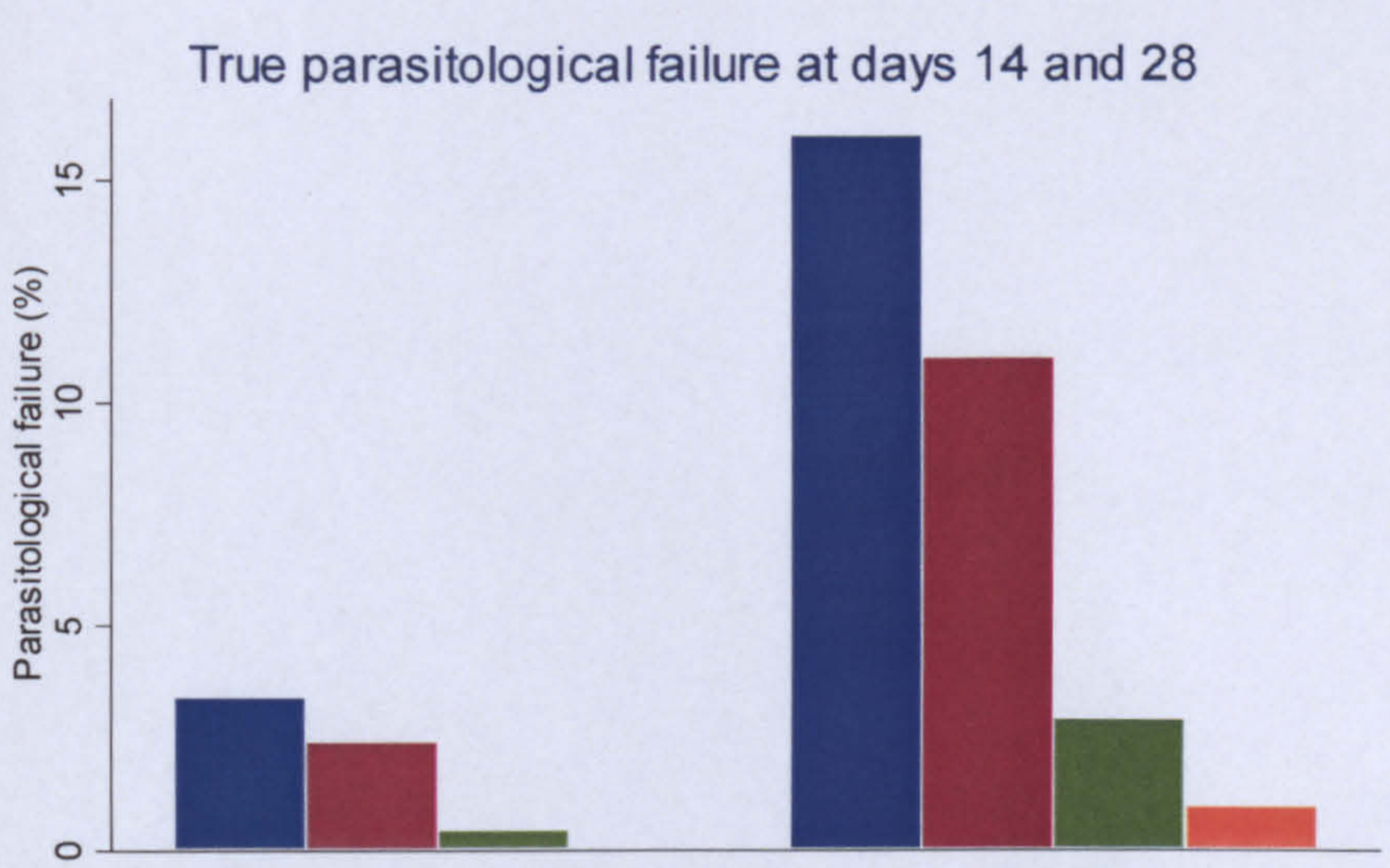


Figure 5.3: PCR-corrected parasitological failure at days 14 and 28 according to treatment group.

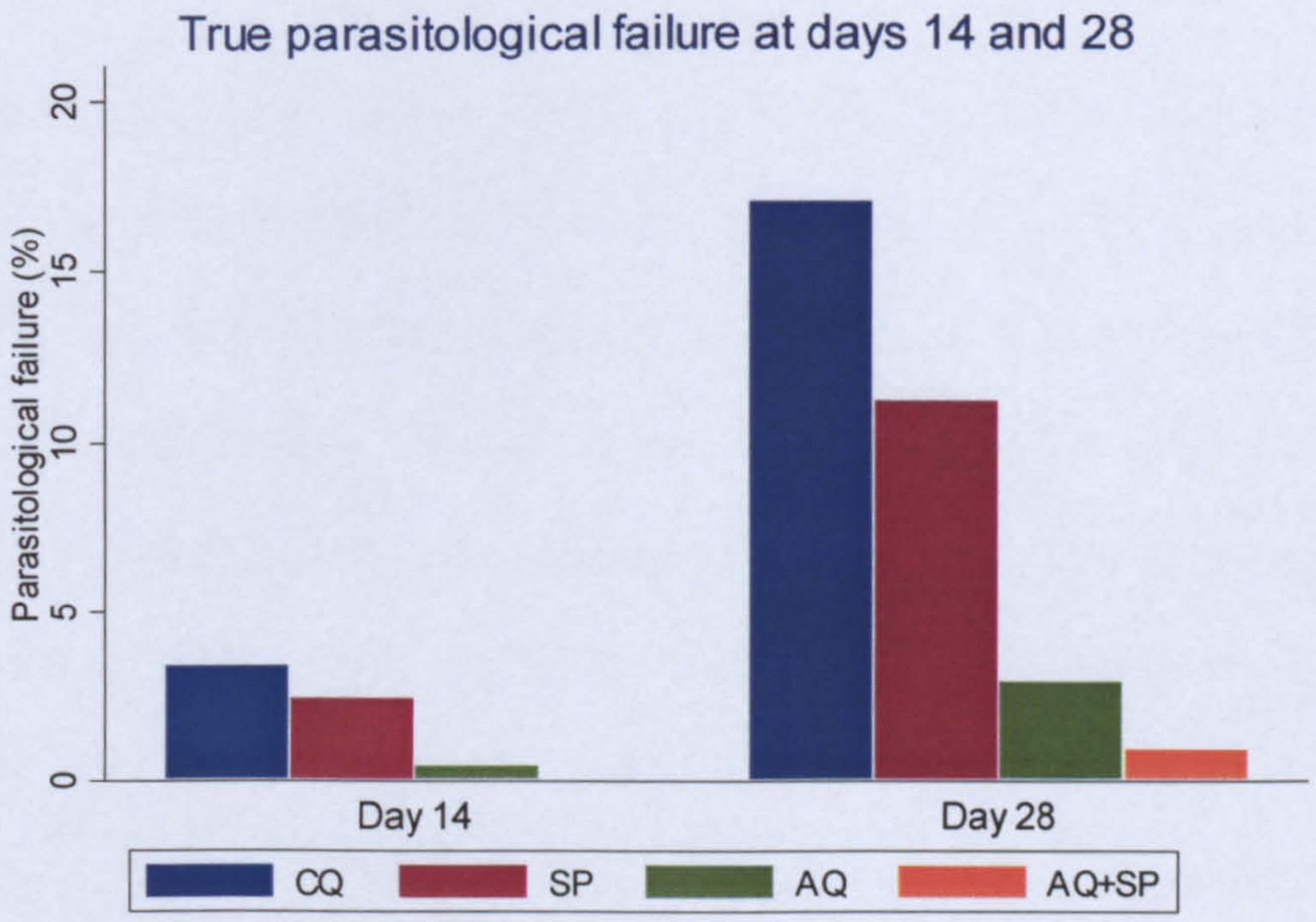


The proportions of parasitological failure by day 14 and 28 when PCR classified re-infections and unclassified results are regarded as withdrawals as recommended by the WHO (WHO 2003b), are shown in **Table 5.2** and **Figure 5.4.** The proportions are generally similar to those shown in **Table 5.1** when re-infections were classified as treatment successes.

Table 5.2 : - PCR-corrected parasitological failure at days 14 and 28 excluding re-infections.

	Corrected parasitological response				
	Success		Failure		Total
	n	(%)	n	(%)	N
DAY 14					
CQ	197	(96.6)	7	(3.4)	204
SP	200	(97.6)	5	(2.4)	205
AQ	212	(99.5)	1	(0.5)	213
AQ+SP	210	(100)	0	(0)	210
Total	819	(98.4)	13	(1.6)	832
DAY 28					
CQ	146	(82.9)	30	(17.1)	176
SP	174	(88.8)	22	(11.2)	196
AQ	196	(97)	6	(3)	202
AQ+SP	205	(99)	2	(1)	207
Total	721	(92.3)	60	(7.7)	781

Figure 5.4: - PCR-corrected parasitological failure at days 14 and 28 excluding re-infections.



The odds of parasitological failure in the treatment groups compared to the CQ group by days 14 and 28 are shown in **Table 5.3.**

Table 5.3: The odds of parasitological failure at days 14 and 28.

	Unadjusted odds ratio (95% CI)		*p-value*	Adjusted odds ratio (95% CI)		*p-value*
DAY 14						
Uncorrected Failure						
CQ	1.0			1.0		
SP	0.7	(0.3 - 1.5)	0.3	0.7	(0.3 - 1.6)	0.4
AQ	0.2	(0.1 - 0.7)	0.01	0.2	(0.1 - 0.7)	0.01
AQ+SP	0.1	(0.01 - 0.5)	0.01	0.1	(0.01 - 0.5)	0.01
Corrected Failure						
CQ	1.0			1.0		
SP	0.7	(0.2 - 2.3)	0.6	0.8	(0.2 - 2.8)	0.7
AQ	0.1	(0.02 - 1.1)	0.06	0.2	(0.02 - 1.5)	0.10
AQ+SP	-	-	-	-	-	-
DAY 28						
Uncorrected Failure						
CQ	1.0			1.0		
SP	0.5	(0.3 - 0.8)	0.002	0.5	(0.3 - 0.8)	0.002
AQ	0.2	(0.1 - 0.3)	< 0.001	0.2	(0.1 - 0.4)	< 0.001
AQ+SP	0.1	(0.02 - 0.1)	< 0.001	0.1	(0.02 - 0.1)	< 0.001
Corrected Failure						
CQ	1.0			1.0		
SP	0.5	(0.3 - 0.9)	0.02	0.7	(0.4 - 1.3)	0.20
AQ	0.2	(0.1 - 0.4)	< 0.001	0.2	(0.1 - 0.4)	< 0.001
AQ+SP	0.1	(0.02 - 0.2)	< 0.001	0.1	(0.01 - 0.2)	< 0.001

SP compared to CQ decreased the risk of uncorrected parasitological failure in the study women by 30% on day 14 (OR=0.7; 95% CI 0.3 to 1.5) and by 50% on day 28 (OR=0.5; 95% CI, 0.3 to 0.8). The ORs after adjusting for baseline parasite density, baseline Hb, parity, gravidity, and prior ill health did not change. After correcting for new infections, SP compared to CQ decreased the risk of parasitological failure in the study women again by 30% on day 14 (OR=0.7; 95% CI 0.2 to 2.3) and by 50% on day 28 (OR=0.4; 95% CI, 0.1 to 1.4). The corrected day 14 OR did not change after adjusting for baseline parasite density, baseline Hb, parity, gravidity, and prior ill health however, the day 28 adjusted OR increased but was no longer significant.

AQ compared to CQ decreased the risk of uncorrected parasitological failure in the study women by 80% on day 14 (OR=0.2; 95% CI, 0.1 to 0.7) and by a similar margin on day 28 (OR=0.2; 95% CI, 0.1 to 0.3). The ORs were 0.2 (95% CI, 0.1 to 0.7) on day 14 and 0.2 (95% CI, 0.1 to 0.4) on day 28 after adjusting for baseline parasite density, baseline Hb, parity or prior ill health. After correcting for new infections, AQ compared to CQ, decreased the risk of parasitological failure in the study women by 90% on day 14 (OR=0.1; 95% CI, 0.02 to 1.1) and by 80% on day 28 (OR=0.2; 95% CI, 0.01 to 0.4). The corrected ORs were 0.2 (95% CI, 0.02 to 1.5) on day 14 and 0.2 (95% CI, 0.01 to 0.4) on day 28 after adjusting for baseline parasite density, baseline Hb, parity or prior ill health.

The AQ+SP combination compared to CQ decreased the risk of uncorrected parasitological failure in the study women by more than 90% on day 14 (OR=0.1; 95% CI, 0.01 to 0.5) and by similar margin on day 28 (OR=0.1; 95% CI, 0.02 to 0.1). These did not change after adjusting for baseline parasite density, baseline Hb, parity, and prior ill health. There were no true parasitological failures on day 14 in the AQ+SP combination group. After correcting for new infections, the AQ+SP combination decreased the risk of parasitological failure by 90% on day 28 (OR=0.1; 95% CI, 0.02 to 0.2). This did not change after adjusting for baseline parasite density, baseline Hb, parity, and prior ill health.

5.3.1 Risk factors for parasitological failure

The unadjusted and adjusted estimates of the association between baseline factors and parasitological failure at day 28 independent of treatment are shown in **Table 5.4**.

As shown in **Table 5.4,** higher parasite densities at enrolment increased the risk of parasitological failure at day 28 ($p<0.001$). Women with parasite densities of 1000/μl or above at enrolment were three times more likely to have parasitological failure by day 28 (OR=3.0; 95% CI, 2.0 to 4.5) compared to women who had parasite densities less than 1000/μl at enrolment. After adjusting for parity, gravidity, baseline Hb and age, women with parasite densities of 1000/μl or above at enrolment were about two and half times more likely to have parasitological failure by day 28 (OR=2.4; 95% CI; 1.6 to 3.8).

Parity was associated significantly with parasitological failure at day 28, ($p<0.001$). Compared to multiparous women, nulliparous and primiparous women respectively were about 8 times (OR=7.7; 95% CI, 3.3 to 17.9) and 3 times (OR=3.1; 95% CI, 1.2 to 8.1) more likely to experience parasitological failure at day 28. After adjusting for baseline parasite density, baseline Hb and age, nulliparous women (OR=8.1; 95% CI, 2.9 to 9.3) and primiparous women (OR=3.2; 95% CI, 1.1 to 9.3) were still more likely to experience parasitological failure at day 28 compared to multiparous women.

Gravidity was associated significantly with parasitological failure at day 28, $p<0.001$. Compared to multigravid women, primigravidaes and secundigravidaes respectively, were about 6 times (OR=6.2; 95% CI, 2.6 to 14.6) and two and half (OR=2.4; 95% CI, 0.9 to 6.3) times more likely to experience parasitological failure at day 28. When both parity and gravidity are included in the same model the effect of gravidity is completely suppressed and that of parity increased. Parity and gravidity are highly correlated with a correlation coefficient of 0.98; $p<0.001$ and so gravidity was not included in the final model.

Baseline haemoglobin was associated significantly with parasitological failure at day 28, p=0.01. Compared to women who had Hb of 11g/dl or above, women with baseline Hb concentrations below 8g/dl were about 4 times (OR=3.7; 95% CI, 1.4 to 9.9) and those with an Hb concentration of 8 to 10.9g/dl, were about 3 times (OR=2.7; 95% CI, 1.1 to 6.9) more likely to experience parasitological failure at day 28. When adjustment is made for baseline parasite density, parity and age, having an Hb concentration below 11g/dl was no longer associated with parasitological failure at day 28 (p=0.1). When the women were categorised as those with an Hb concentrations 5 to 10.9g/dl or, 11g/dl or above, women with baseline Hb concentrations 5 to 10.9g/dl were about 3 times more likely to experience parasitological failure compared to those with an Hb concentration of 11g/dl or above at enrolment (OR=2.9; 95% CI, 1.2 to 7.3 p<0.02).

Younger mothers were more likely to experience parasitological failure at 28 (p=0.01) but after adjusting for baseline parasite density, baseline Hb and parity age was no longer associated with parasitological failure at day 28 (p=0.1).

Table 5.4: - Baseline factors associated with parasitological failure at day 28.

	Success (N=721)		Failure (N=117)		Unadjusted odds ratio (95% CI)		LR test	Adjusted odds ratio (95% CI)		LT test
	n	(%)	n	(%)						
Baseline parasite density										
< 1000/ul	411	(57)	36	(30.8)	1.0		**p<0.001**	1.0		**p<0.001**
>/=1000/ul	310	(43)	81	(69.2)	3.0	(2.0 - 4.5)		2.4	(1.6 - 3.8)	
Parity										
2 or above	183	(25.4)	6	(5.1)	1.0		**p<0.001**	1.0		**p<0.001**
1	166	(23)	17	(14.5)	3.1	(1.2 - 8.1)		3.2	(1.1 - 9.3)	
0	372	(51.6)	94	(80.3)	7.7	(3.3 - 17.9)		8.1	(2.9 - 22.5)	
Gravidity										
3 or above	195	(27)	8	(6.8)	1.0		**p<0.001**			
2	173	(24)	18	(15.4)	2.5	(1.1 - 6.0)				
1	353	(49)	91	(77.8)	6.3	(3.0 - 13.2)				
Baseline Haemoglobin										
>/=11g/dl	83	(11.5)	5	(4.3)	1.0		**p=0.01**	1.0		**p=0.1**
8 - 10.9 g/dl	504	(69.9)	82	(70.1)	2.7	(1.1 - 6.9)		2.6	(1.0 - 6.9)	
<8g/dl	134	(18.6)	30	(25.6)	3.7	(1.4 - 9.9)		2.6	(0.9 - 7.2)	
Age [years]										
30+	104	(14.4)	7	(6)	1.0		**p=0.01**	1.0		**p=0.7**
25-29	136	(18.9)	16	(13.7)	1.7	(0.7 - 4.4)		1.0	(0.3 - 2.8)	
20-24	262	(36.3)	45	(38.5)	2.6	(1.1 - 5.8)		0.7	(0.2 - 1.8)	
<20	219	(30.4)	49	(41.9)	3.3	(1.5 - 7.6)		0.7	(0.2 - 1.9)	

The unadjusted and adjusted estimates of the association between baseline factors and parasitological failure at day 14 independent of treatment are shown in **Table 5.5.**

By day 14, baseline parasite density was associated significantly with parasitological failure ($p<0.001$) as shown in **Table 5.5.** Women with parasite densities of 1000/μl or above at enrolment were more likely to fail treatment at day 14 compared to women who had parasite densities less than 1000/μl at enrolment (OR=7.6; 95% CI, 2.6 to 22.1). After adjustments were made for baseline Hb, parity and age high enrolment parasite density was still associated significantly with parasitological failure at day 14 (<0.001). In the univariate analysis, parity and gravidity were associated significantly with parasitological failure at day 14 but not after adjustments were made for age. Age and baseline Hb were not associated with parasitological failure at day 14 ($p>0.1$).

Table 5.5: - Baseline factors associated with parasitological failure at day 14.

	Success (N=819)		Failure (N=29)		Unadjusted odds ratio (95% CI)		LR test	Adjusted odds ratio (95% CI)		LT test
	n	(%)	n	(%)						
Baseline parasite density										
< 1000/ul	450	(54.9)	4	(13.8)	1.0		**p<0.001**	1.0		**p<0.001**
>/=1000/ul	369	(45.1)	25	(86.2)	7.6	(2.6 - 22.1)		6.5	(2.2 - 19.2)	
Parity										
2 or above	191	(23.3)	2	(6.9)	1.0		**p=0.03**	1.0		**p=0.1**
1	181	(22.1)	5	(17.2)	2.6	(0.5 - 13.8)		2.7	(0.4 - 18.1)	
0	447	(54.6)	22	(75.9)	4.7	(1.1 - 20.2)		5.0	(0.8 - 29.3)	
Gravidity										
3 or above	203	(24.8)	2	(6.9)	1.0		**p=0.02**			
2	191	(23.3)	5	(17.2)	2.7	(0.5 - 13.8)				
1	425	(51.9)	22	(75.9)	5.3	(1.2 - 22.6)				
Baseline Haemoglobin										
>/=11g/dl	88	(10.7)	3	(10.3)	1.0		**p=0.9**	1.0		**p=0.7**
8 - 10.9 g/dl	571	(69.7)	21	(72.4)	1.1	(0.3 - 3.7)		0.9	(0.3 - 3.3)	
<8g/dl	160	(19.5)	5	(17.2)	0.9	(0.2 - 3.9)		0.6	(0.1 - 2.8)	
Age [years]										
30+	110	(13.4)	3	(10.3)	1.0		**p=0.6**	1.0		**p=0.4**
25-29	149	(18.2)	3	(10.3)	0.7	(0.1 - 3.7)		0.4	(0.1 2.5)	
20-24	298	(36.4)	13	(44.8)	1.6	(0.4 - 5.7)		0.5	(0.1- 2.4)	
<20	262	(32)	10	(34.5)	1.4	(0.4 - 5.2)		0.4	(0.1 - 2.0)	

5.4 Haematological outcomes

The effect of treatment on haemoglobin was assessed by determining the absolute change from baseline over time using ANOVA and multiple regression analysis. The proportions of pregnant women with 3 categories of haemoglobin concentrations at days 14 and 28 after the start of treatment are shown in **Table 5.6**.

Table 5.6: - Haemoglobin concentrations at days 14 and 28 after start of treatment.

	CQ		SP		AQ		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	N	(%)
DAY 14										
< 8g/dl	21	(10.1)	38	(18.1)	18	(8.5)	31	(14.7)	108	(12.8)
8 - 10.9g/dl	153	(73.2)	143	(68.1)	167	(78.8)	145	(68.7)	608	(72.2)
11g/dl or above	35	(16.8)	29	(13.8)	27	(12.7)	35	(16.6)	126	(15)
Mean (SD)	9.7	(1.3)	9.4	(1.4)	9.6	(1.3)	9.6	(1.6)	9.6	(1.4)
Median (IQR)	9.7	(1.6)	9.5	(1.9)	9.8	(1.6)	9.6	(1.9)	9.6	(1.8)
DAY 28										
< 8g/dl	26	(12.7)	17	(8.3)	7	(3.3)	10	(4.8)	60	(7.2)
8 - 10.9g/dl	130	(63.4)	133	(64.9)	150	(70.1)	141	(67.8)	554	(66.6)
11g/dl or above	49	(23.9)	55	(26.8)	57	(26.6)	57	(27.4)	218	(26.2)
Mean (SD)	9.8	(1.7)	10.0	(1.6)	10.2	(1.4)	10.2	(1.4)	10.1	(1.5)
Median (IQR)	9.8	(2.1)	9.9	(2)	10.0	(1.9)	10.1	(2)	10.0	(2)

As shown in **Table 5.6** and **Figure 5.5,** there was a general improvement in Hb levels within all treatment groups over the follow up period after treatment. The overall proportions of women with Hb below 8g/dl had decreased from about 20% on day 0 to 13% and 7% on days 14 and 28 respectively while the proportions of women with an Hb of 11g/dl or above had increased from 11% on day 0 to 15% and 26% on days 14 and 28 respectively. Also within all the treatment groups mean haemoglobin concentrations increased by days 14 and 28 respectively compared to the means at enrolment as shown in **Figure 5.6**.

Figure 5.5: - Improvements in haemoglobin concentrations following treatment.

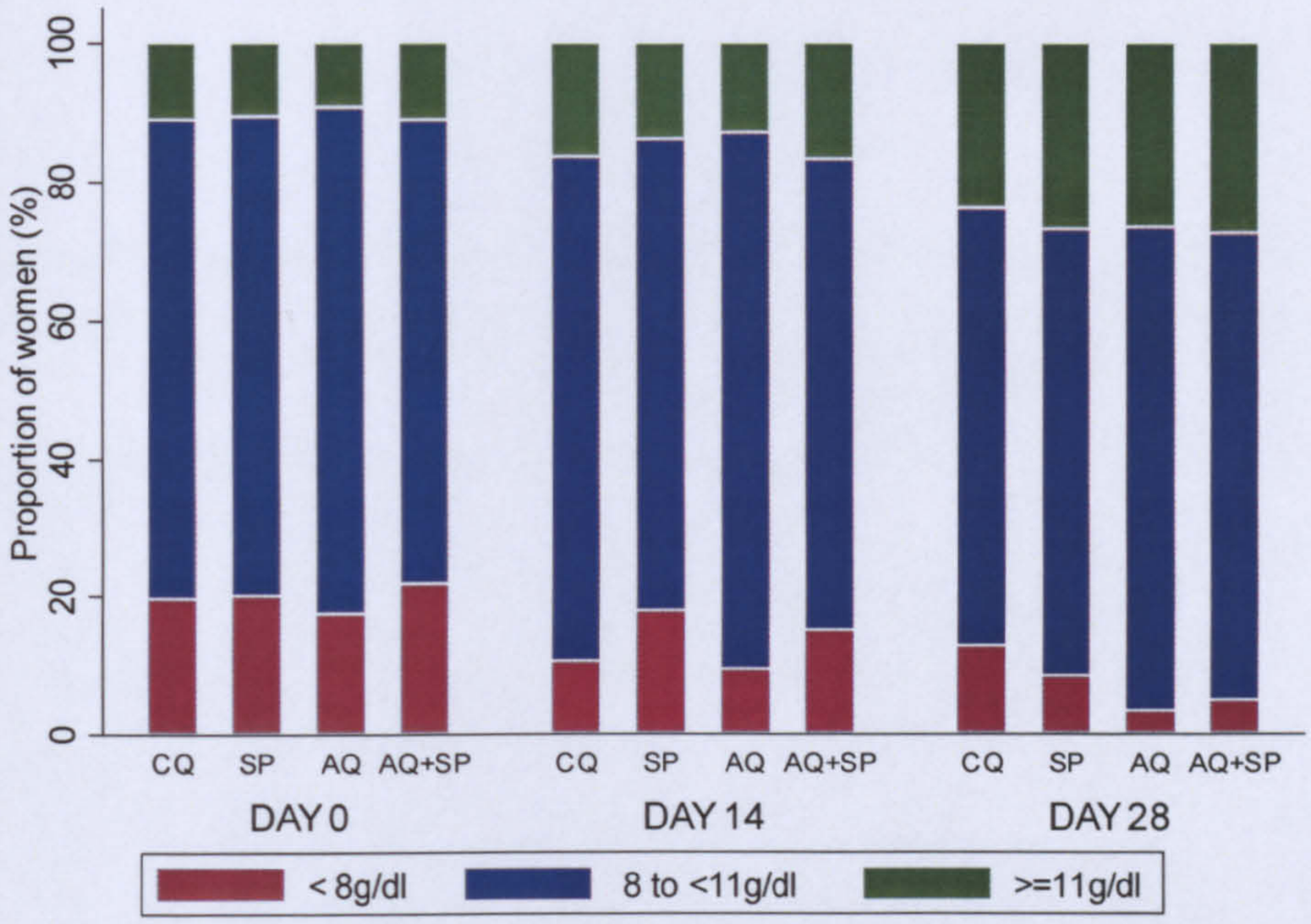
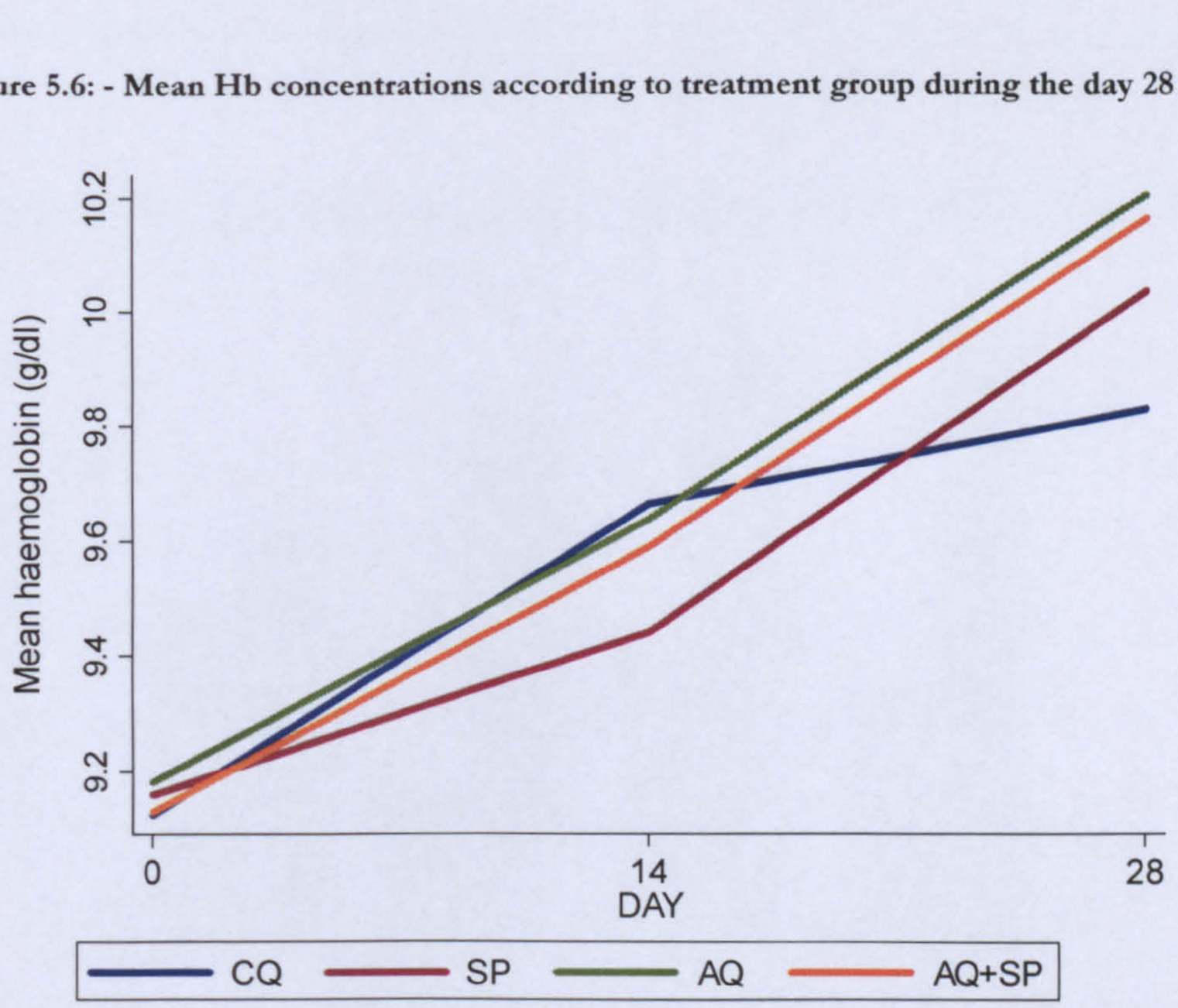


Figure 5.6: - Mean Hb concentrations according to treatment group during the day 28 follow up period

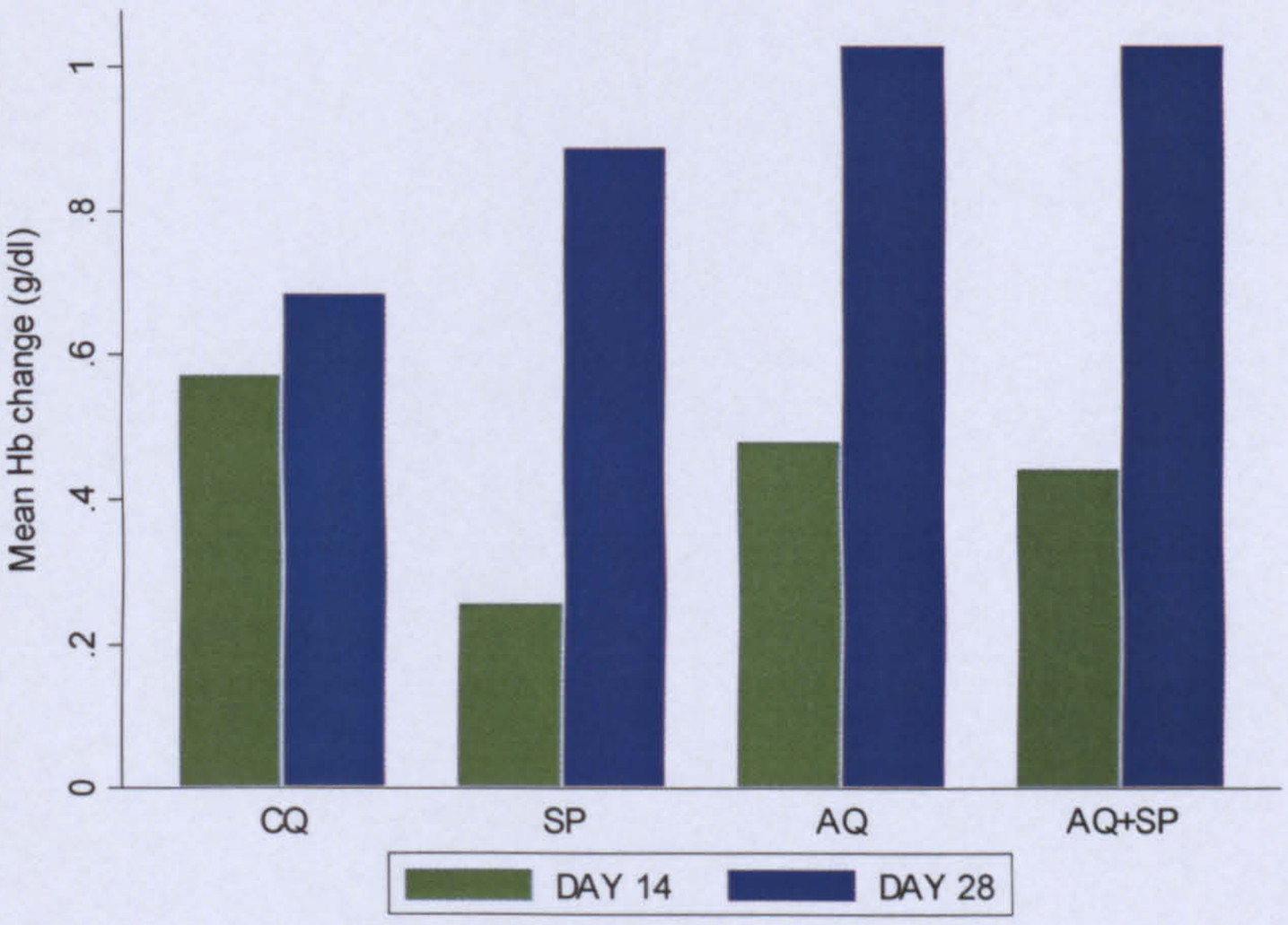


The mean changes in haemoglobin concentrations within and between the treatment groups over time are shown in **Table 5.7 and Figure 5.7**

Table 5.7: - Comparison of mean haemoglobin changes by AQ, SP and AQ+SP with CQ.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p – value)
DAY 14							
CQ	0.6	(1.4)	(-4.0 to 5.6)				1.9 (0.1)
SP	0.3	(1.3)	(-4.4 to 4.6)	-0.3	(-0.6 to -0.1)	0.02	
AQ	0.5	(1.4)	(-6.4 to 4.9)	-0.1	(-0.4 to 0.2)	0.5	
AQ+SP	0.4	(1.4)	(-5.0 to 6.6)	-0.1	(-0.4 to 0.1)	0.3	
DAY 28							
CQ	0.7	(1.6)	(-3.1 to 6.0)				2.5(0.06)
SP	0.9	(1.4)	(-4.5 to 6.5)	0.2	(-0.1 to 0.5)	0.2	
AQ	1.0	(1.4)	(-6.0 to 5.4)	0.3	(0.1 to 0.6)	0.02	
AQ+SP	1.0	(1.5)	(-5.8 to 7.7	0.3	(0.1 to 0.6)	0.02	

Figure 5.7: - Change in haemoglobin levels over 28 days follow up period after start of treatment.



There was an overall mean increase of 0.4g/dl (95% CI; 0.3 to 0.5) p<0.001 in Hb concentration at day 14 relative to the baseline mean concentrations. The mean changes were 0.6, 0.3, 0.5 and 0.4g/dl respectively for CQ, SP, AQ and AQ+SP combination groups over the initial 14 days following enrolment. There was a mean increase in haemoglobin concentration of about 0.1g/dl more in the CQ group than in the AQ and AQ+SP groups respectively and about 0.3g/dl more in the CQ group than in the SP group. However, the differences between the CQ and AQ and the AQ+SP groups were not statistically significant while the difference for the SP group was significant (p = 0.02). The absolute mean changes were statistically not different at day 14 (F-statistic 1.9; p=0.1)

At day 28, the overall mean increase in Hb concentration was 0.9g/dl (95% CI; 0.8 to 1.0) p<0.001 relative to the baseline mean concentrations. The mean changes were 0.7, 0.9, 1.0 and 1.0g/dl respectively for CQ, SP, AQ and AQ+SP combination groups at day 28. The mean haemoglobin increase was about 0.3g/dl less in the CQ group compared to the AQ and AQ+SP groups respectively and about 0.2g/dl less in the CQ group than in the SP group. These differences were statistically significant for the CQ versus AQ and AQ+SP comparisons (p = 0.02) but not significant in the case of CQ versus SP (p = 0.2). The mean changes in haemoglobin remained unchanged when baseline parasite density, parity, gravidity or age were included in the regression model. As shown in **Figure 5.7,** within each treatment group, the change at day 28 tended to be higher than would have occurred by day 14.

CHAPTER SIX

SAFETY AND TOLERANCE

CHAPTER 6 SAFETY AND TOLERANCE

6.1 Introduction

This chapter presents the results of the safety and tolerance observations made among the study pregnant women over the 28-day follow up period after the start of treatment. The first section describes the types and incidence of adverse effects reported by women during post treatment follow up at days 3, 7, 14 and 28 and makes comparisons between treatment groups. A comparison is also made of the incidences of complaints obtained from a sample of untreated non-parasitaemic pregnant women screened at the same clinic and who received the same number of follow up visits with those of enrolled parasitaemic pregnant women. The second section describes the levels of liver enzymes and bilirubin at days 0, 14 and 28 and compares: -

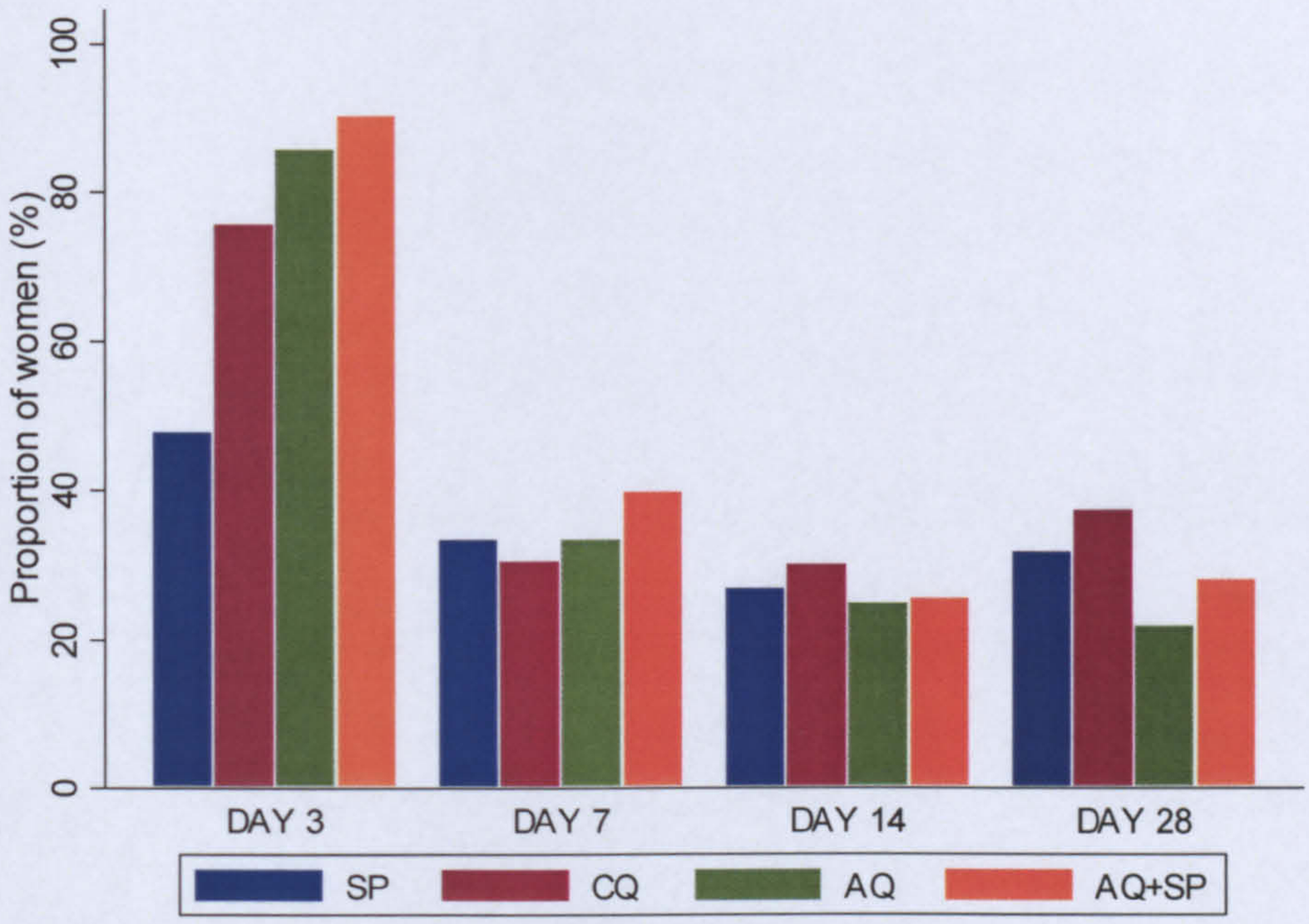
- The proportions of women within predefined categories within treatment groups and in all treatment groups compared with CQ.
- The absolute mean changes at days 14 and 28 over baseline levels within treatment groups and in all treatment groups compared with CQ.
- The baseline liver enzymes and bilirubin levels in the study pregnant women with values obtained from a random sample of pregnant women who had negative OptiMAL® dipstick tests at screening.

This section also compares the white blood cell counts at days 0, 3, 7, 14 and 28 based on a reference range of 4.0 - 11.0 x 10^9/L within treatment groups and in all treatment groups compared with the CQ group.

6.2 Incidence of drug adverse events

The proportions of treated pregnant women who reported an adverse effect at days 3, 7, 14 and 28 of follow up are shown in **Figure 6.1.**

Figure 6.1: - Reports of a adverse effect on follow up days according to treatment group



Overall, seventy-five percent of women at follow up day 3 reported an adverse effect. As shown in **Figure 6.1** and **Table 6.1**, adverse effects were reported by 76%, 86% and 90% of women in the CQ, AQ and AQ+SP groups respectively compared to 48% by women in the SP group. General weakness, dizziness, vomiting, itching and nausea were the five most frequently reported adverse effects. The reports of weakness, vomiting, dizziness and nausea were highest in the AQ and AQ+SP groups and least in the SP groups. The reports of itching were highest in the CQ group and least in the SP group. A total of 75 women at day 3 had not completed the last dose

of their treatment; 40(53%) claimed they had forgotten to take the last dose and 35(47%) attributed their inability to finish their treatment to adverse effects.

Table 6.1: - Reports of adverse effects by study pregnant women at day 3 after the start of treatment.

	CQ		AQ		SP		AQ+SP		Total		p-value
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
Report of a adverse effect											
No	52	(24.2)	31	(14.1)	113	(52.1)	21	(9.7)	217	(25)	
Yes	163	(75.8)	189	(85.9)	104	(47.9)	196	(90.3)	652	(75)	<0.001
General weakness											
No	109	(50.7)	35	(15.9)	153	(70.5)	35	(16.1)	332	(38.2)	
Yes	106	(49.3)	185	(84.1)	64	(29.5)	182	(83.9)	537	(61.8)	<0.001
Dizziness											
No	118	(54.9)	93	(42.3)	184	(84.2)	95	(43.8)	490	(56.4)	
Yes	97	(45.1)	127	(57.7)	33	(15.2)	122	(56.2)	379	(43.6)	<0.001
Vomiting											
No	145	(67.4)	119	(54.1)	185	(85.3)	86	(39.6)	535	(61.6)	
Yes	70	(32.6)	101	(45.9)	32	(14.7)	131	(60.4)	334	(38.4)	<0.001
Itching											
No	127	(59.1)	149	(67.7)	182	(83.9)	161	(74.2)	619	(71.2)	
Yes	88	(40.9)	71	(32.3)	35	(16.1)	56	(25.8)	250	(28.8)	<0.001
Nausea											
No	165	(76.7)	163	(74.1)	191	(88)	135	(62.2)	654	(75.3)	
Yes	50	(23.3)	57	(25.9)	26	(12)	82	(37.8)	215	(24.7)	<0.001
Headache											
No	169	(78.6)	172	(78.2)	172	(79.3)	163	(75.1)	676	(77.8)	
Yes	46	(21.4)	48	(21.8)	45	(20.7)	54	(24.9)	193	(22.2)	0.74
Completed treatment											
No	22	(10.2)	15	(6.8)	13	(6)	25	(11.5)	75	(8.6)	
Yes	193	(89.8)	205	(93.2)	204	(94)	192	(88.5)	794	(91.4)	0.12
Why treatment not completed											
Forgot	9	(40.9)	9	(60)	12	(100)	10	(38.5)	40	(53.3)	
Adverse effects	13	(59.1)	6	(40)	0	(0)	16	(61.5)	35	(46.7)	0.002
Routine activities interfered											
No	189	(87.9)	133	(60.4)	208	(95.8)	124	(57.1)	654	(75.3)	
Yes	26	(12.1)	87	(39.6)	9	(4.2)	93	(42.9)	215	(24.7)	<0.001

For women in the SP group, the active drugs were taken on day 0 supervised so the drug pack they took home contained only placebo. However, no woman in the SP group who had not finished taking the contents of this pack attributed it to adverse effects. Twenty-five percent of the women at day 3 claimed their routine activities had been inhibited by the effects of the study drugs. This was reported by 43%, 40%, 12% and 4% of women in the AQ+SP, AQ, CQ and SP groups respectively.

A comparison of the SP, AQ and AQ+SP groups with the CQ group with respect to the incidence of common adverse effects among the study group is shown in **Table 6.2.**

The odds of reporting an adverse effect following treatment with AQ were two times higher compared with those treated with CQ; (OR=1.9, 95% CI, 1.7 - 3.2 p=0.01). Those who received the AQ+SP combination were 3 times more likely to report an adverse effect (OR=3.0; 95% CI, 1.7 - 5.1 p<0.001) compared to the CQ group. Reports of an adverse effect were 70% less frequently from those who took SP (OR=0.3; 95% CI, 0.19 - 0.44 p<0.001) compared with those who took CQ.

Reports of general weakness were 5 times more frequent from the AQ (OR=5.4; 95% CI, 3.5 to 8.5 p<0.001) and AQ+SP (OR=5.3; 95% CI, 3.5 to 8.4 p<0.001) groups respectively and about 60% less frequent from the SP group (OR=0.4; 95% CI, 0.3 to 0.6 p<0.001) compared with the CQ group. Reports of vomiting were more frequent from the AQ (OR=1.8; 95% CI, 1.2 to 2.6 p<0.001) and AQ+SP (OR=3.2; 95% CI, 2.1 to 4.7) groups respectively and 65% less frequent from the SP group (OR=0.3; 95% CI, 0.3 to 0.7 p<0.001) compared with the CQ group. AQ alone was not associated with nausea (p=0.5) however, reports of nausea were 2 times more frequent in the AQ+SP combination group (OR=2.0; 95% CI, 1.3 to 3.0 p=0.001) and about 50% less frequent in the SP group (OR=0.5; 95% CI, 0.3 to 0.8 p=0.002) compared with the CQ group. Reports of dizziness were about one and half times more frequent in the AQ (OR=1.7;

95% CI, 1.1 to 2.4 p=0.01) and AQ+SP (OR=1.6; 95% CI, 1.1 to 2.3 p=0.02) groups respectively; and about 80% less frequent in the SP group (OR=0.2; 95% CI, 0.1 to 0.4 p<0.001). Itching was most frequently reported by those in the CQ group and least frequent in the SP group (OR=0.3; 95% CI, 0.2 to 0.4 p<0.001). Reports of inability to perform routine chores following treatment were five times more likely to come from women who took AQ (OR=4.8; 95% CI, 2.9 to 7.8 p<0.001) and AQ+SP (OR=5.5; 95% CI, 3.3 to 8.9 p<0.001) respectively and about 70% less frequent from those who took SP (OR=0.3; 95% CI, 0.1 to 0.7 p=0.004) compared to taking CQ. The frequency of reports of headache were similar in all the treatment groups (p>0.1). When adjustments were made for baseline parasite density, baseline Hb, parity, gestation, age and prior ill health, the odds ratios were similar to the unadjusted estimates.

Table 6.2: - Odds of adverse effects in the SP, AQ and AQ+SP groups compared to the CQ group.

	CQ	SP		p - value	AQ		p-value	AQ+SP		p-value
Report of a adverse effect										
Unadjusted odds ratio (95% CI)		0.3	(0.2 - 0.4)	<0.001	1.9	(1.2 - 3.2)	0.01	3.0	(1.7 - 5.1)	<0.001
Adjusted odds ratio (95% CI)**	1.0	0.3	(0.2 - 0.4)	<0.001	2.0	(1.2 - 3.3)	0.01	2.9	(1.7 - 5.1)	<0.001
General weakness										
Unadjusted odds ratio (95% CI)		0.4	(0.3 - 0.6)	<0.001	5.4	(3.5 - 8.5)	<0.001	5.3	(3.4 - 8.4)	<0.001
Adjusted odds ratio (95% CI)	1.0	0.4	(0.3 - 0.6)	<0.001	5.5	(3.5 - 8.6)	<0.001	5.3	(3.4 - 8.4)	<0.001
Vomiting										
Unadjusted odds ratio (95% CI)		0.3	(0.3 - 0.7)	<0.001	1.8	(1.2 - 2.6)	0.01	3.2	(2.1 - 4.7)	<0.001
Adjusted odds ratio (95% CI)	1.0	0.4	(0.3 - 0.6)	<0.001	1.7	(1.2 - 2.6)	0.01	3.3	(2.2 - 5.0)	<0.001
Nausea										
Unadjusted odds ratio (95% CI)		0.5	(0.3 - 0.8)	0.002	1.2	(0.7 - 1.8)	0.5	2.0	(1.3 - 3.0)	0.001
Adjusted odds ratio (95% CI)	1.0	0.4	(0.3 - 0.8)	0.002	1.2	(0.7 - 1.8)	0.5	2.0	(1.3 - 3.1)	0.002
Dizziness										
Unadjusted odds ratio (95% CI)		0.2	(0.1 - 0.4)	<0.001	1.7	(1.1 - 2.4)	0.01	1.6	(1.1 - 2.3)	0.02
Adjusted odds ratio (95% CI)	1.0	0.2	(0.1 - 0.4)	<0.001	1.7	(1.2 - 2.6)	0.01	1.6	(1.1 - 2.3)	0.03
Itching										
Unadjusted odds ratio (95% CI)		0.3	(0.2 - 0.4)	<0.001	0.7	(0.5 - 1.0)	0.06	0.5	(0.3 - 0.7)	0.001
Adjusted odds ratio (95% CI)	1.0	0.3	(0.2 - 0.4)	<0.001	0.7	(0.5 - 1.0)	0.07	0.5	(0.3 - 0.8)	0.001
Routing activity										
Unadjusted odds ratio (95% CI)		0.3	(0.1 – 0.7)	0.004	4.8	(2.9 – 7.8)	<0.001	5.5	(3.3 – 8.9)	<0.001
Adjusted odds ratio (95% CI)	1.0	0.3	(0.1 – 0.7)	0.004	5.2	(3.2 – 8.7)	<0.001	6	(3.6 – 9.8)	<0.001
Headache										
Unadjusted odds ratio (95% CI)		1.0	(0.6 - 1.5)	0.9	1.0	(0.6 - 1.6)	0.9	1.2	(0.8 - 1.9)	0.4
Adjusted odds ratio (95% CI)	1.0	1.0	(0.6 - 1.5)	0.8	1.0	(0.7 - 1.7)	0.9	1.2	(0.8 - 1.9)	0.5

** Odds ratios adjusted for baseline parasite density, baseline Hb, parity, prior ill health, gestation and age.

Table 6.3: - Reports of adverse effects by study pregnant women at day 7 after the start of treatment.

	CQ		AQ		SP		AQ+SP		Total		p-value
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
Report of an adverse effect											
No	152	(69.4)	145	(66.5)	145	(66.5)	130	(60.2)	572	(65.7)	
Yes	67	(30.6)	73	(33.5)	73	(33.5)	86	(39.8)	299	(34.3)	0.2
General weakness											
No	170	(77.6)	146	(67)	165	(75.7)	132	(61.1)	613	(70.4)	
Yes	49	(22.4)	72	(33)	53	(24.3)	84	(38.9)	258	(29.6)	<0.001
Dizziness											
No	185	(84.5)	186	(85.3)	194	(89)	171	(79.2)	736	(84.5)	
Yes	34	(15.5)	32	(14.7)	24	(11)	45	(20.8)	135	(15.5)	0.04
Vomiting											
No	188	(85.8)	190	(87.2)	201	(92.2)	176	(81.5)	755	(86.7)	
Yes	31	(14.2)	28	(12.8)	17	(7.8)	40	(18.5)	116	(13.3)	0.01
Itching											
No	201	(91.8)	191	(87.6)	190	(87.2)	193	(89.3)	775	(89)	
Yes	18	(8.2)	27	(12.4)	28	(12.8)	23	(10.7)	96	(11)	0.4
Nausea											
No	186	(84.9)	195	(89.4)	198	(90.8)	183	(84.7)	762	(87.5)	
Yes	33	(15.1)	23	(10.6)	20	(9.2)	33	(15.3)	109	(12.5)	0.1
Headache											
No	185	(84.5)	189	(86.7)	176	(80.7)	183	(84.7)	733	(84.2)	
Yes	34	(15.5)	29	(13.3)	42	(19.3)	33	(15.3)	138	(15.8)	0.4
Routine activities interfered											
No	4	(1.8)	16	(7.3)	2	(0.9)	16	(7.4)	38	(4.4)	
Yes	215	(98.2)	202	(92.7)	216	(99.1)	200	(92.6)	833	(95.6)	<0.001

Overall, thirty-four percent of women at follow up day 7 reported an adverse effect. As shown in **Figure 6.1** and **Table 6.3**, the frequencies of reports of an adverse effect, itching, nausea and headache similar in all the treatment groups ($p<0.1$). General weakness was more frequently reported by women in the AQ (33%) and AQ+SP (39%) groups than by women in the CQ (22%) and SP (24%) groups. Dizziness was reported most frequently by women in the AQ+SP (21%) compared to 16%, 15% and 11% of women in the CQ, AQ and SP the groups respectively. Vomiting was reported less frequently by women in the SP (8%) group compared to 14%, 13% and 19% of women in the CQ, AQ and AQ+SP groups respectively. Four percent of the women at day 7 claimed their routine activities had been inhibited by the effects of the study

drugs. This was reported more frequently by women in the AQ (7%) and AQ+SP (7%) groups compared to 2% and1% of women in the CQ and SP respectively.

A comparison of the SP, AQ and AQ+SP groups with the CQ group with respect to the incidence of common adverse effects reported on day 7 was made using logistic regression. Compared to the CQ group, women in the AQ+SP combination group were one and half times more likely (OR=1.5; 95% CI, 1.0 to 2.2 p=0.04) to report an adverse effect on day 7 but the AQ and SP groups respectively, were not different from the CQ (OR= 1.1; 95% CI, 0.8 to 1.7 p=0.5). Reports of general weakness on day 7 were more frequent from the AQ (OR=1.7; 95% CI, 1.1 to 2.6 p<0.01) and AQ+SP (OR=2.2; 95% CI, 1.5 to 3.6 p<0.001) groups respectively compared with the CQ group but the SP group was not different from the CQ group (OR=1.1; 95% CI, 0.7 to 1.7 p<0.6). Compared to the CQ group, those in the SP group were 50% less likely to report vomiting on day 7 (OR=0.5; 95% CI, 0.3 to 1.0 p=0.03) but there was no statistical difference in incidence in the AQ and AQ+SP groups respectively compared to the CQ group (p>0.1). Although the distribution of reports of dizziness on day 7 according to treatment groups was significantly different (p=0.04), they were not when all the groups were compared to the CQ group (p>0.1). Reports of inability to perform routine chores on day 7 were four times more likely to come from women in the AQ (OR=4.3; 95% CI, 1.4 to 13.0 p=0. 01) and AQ+SP (OR=4.3; 95% CI, 1.4 to 13.1 p<0.01) groups respectively and about 50% less frequent from those in the SP group (OR=0.5; 95% CI, 0.1 to 2.7 p=0.4) compared to those in the CQ group. When adjustments were made for baseline parasite density, baseline Hb, parity, gestation, age and prior ill health, the odds ratios were similar to the unadjusted estimates.

Table 6.4: - Reports of adverse effects by study pregnant women at day 14 after the start of treatment.

	CQ		AQ		SP		AQ+SP		Total		p-value
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
Report of a side effect											
No	151	(69.9)	165	(75)	157	(73)	159	(74.3)	632	(73.1)	
Yes	65	(30.1)	55	(25)	58	(27)	55	(25.7)	233	(26.9)	0.6
General weakness											
No	181	(83.8)	188	(85.4)	171	(79.5)	175	(81.8)	715	(82.7)	
Yes	35	(16.2)	32	(14.6)	44	(20.5)	39	(18.2)	150	(17.3)	0.4
Dizziness											
No	192	(88.9)	199	(90.4)	193	(89.8)	192	(89.7)	776	(89.7)	
Yes	24	(11.1)	21	(9.6)	22	(10.2)	22	(10.3)	89	(10.3)	0.9
Vomiting											
No	197	(91.2)	200	(90.9)	206	(95.8)	200	(93.5)	803	(92.8)	
Yes	19	(8.8)	20	(9.1)	9	(4.2)	14	(6.5)	62	(7.2)	0.2
Itching											
No	205	(94.9)	213	(96.8)	191	(88.8)	207	(96.7)	816	(94.3)	
Yes	11	(5.1)	7	(3.2)	24	(11.2)	7	(3.3)	49	(5.7)	0.001
Nausea											
No	195	(90.3)	202	(91.8)	206	(95.8)	196	(91.6)	799	(92.4)	
Yes	21	(9.7)	18	(8.2)	9	(4.2)	18	(8.4)	66	(7.6)	0.2
Headache											
No	179	(82.9)	183	(83.2)	189	(87.9)	183	(85.5)	734	(84.9)	
Yes	37	(17.1)	37	(16.8)	26	(12.1)	31	(14.5)	131	(15.1)	0.4
Routine activities interfered											
No	3	(1.4)	5	(2.3)	4	(1.9)	2	(0.9)	14	(1.6)	
Yes	213	(98.6)	215	(97.7)	211	(98.1)	212	(99.1)	851	(98.4)	0.7

Overall, twenty-seven percent of women at follow up day 14 reported an adverse effect. As shown in **Figure 6.1** and **Table 6.4**, the frequencies of reports of an adverse effect, general weakness, vomiting, nausea, headache and inhibition of performance of household chores were similar in all the treatment groups ($p<0.1$). Itching was reported most frequently by women in the SP (11%) group compared to 5%, 3% and 3% of women in the CQ, AQ and AQ+SP groups respectively and this difference was significant $p=0.001$. Compared to the CQ group, women in the SP group were about twice likely to report itching on day 14 (OR=2.3; 95% CI, 1.1 to 4.9 $p=0.02$) but there was no statistical difference in incidence in the AQ and AQ+SP groups respectively compared to the CQ group ($p>0.1$).

Table 6.5: - Reports of adverse effects by study pregnant women at day 28 after the start of treatment.

	CQ		AQ		SP		AQ+SP		Total		p-value
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
Report of a side effect											
No	129	(62.6)	168	(78.1)	142	(68.3)	153	(71.8)	592	(70.3)	
Yes	77	(37.4)	47	(21.9)	66	(31.7)	60	(28.2)	250	(29.7)	0.01
General weakness											
No	150	(72.8)	174	(80.9)	163	(78.4)	169	(79.3)	656	(77.9)	
Yes	56	(27.2)	41	(19.1)	45	(21.6)	44	(20.7)	186	(22.1)	0.2
Dizziness											
No	177	(85.9)	197	(91.6)	185	(88.9)	186	(87.3)	745	(88.5)	
Yes	29	(14.1)	18	(8.4)	23	(11.1)	27	(12.7)	97	(11.5)	0.3
Vomiting											
No	186	(90.3)	196	(91.2)	190	(91.3)	187	(87.8)	759	(90.1)	
Yes	20	(9.7)	19	(8.8)	18	(8.7)	26	(12.2)	83	(9.9)	0.6
Itching											
No	186	(90.3)	199	(92.6)	187	(89.9)	196	(92)	768	(91.2)	
Yes	20	(9.7)	16	(7.4)	21	(10.1)	17	(8)	74	(8.8)	0.7
Nausea											
No	178	(86.4)	200	(93)	186	(89.4)	193	(90.6)	757	(89.9)	
Yes	28	(13.6)	15	(7)	22	(10.6)	20	(9.4)	85	(10.1)	0.2
Headache											
No	150	(72.8)	181	(84.2)	166	(79.8)	181	(85)	678	(80.5)	
Yes	56	(27.2)	34	(15.8)	42	(20.2)	32	(15)	164	(19.5)	0.01
Routine activities interfered											
No	4	(1.9)	8	(3.7)	2	(1)	8	(3.8)	22	(2.6)	
Yes	202	(98.1)	207	(96.3)	206	(99)	205	(96.2)	820	(97.4)	0.2

Overall, thirty percent of women at follow up day 28 reported an adverse effect. As shown in **Figure 6.1 and Table 6.5,** the reports of adverse effects from women were more frequent in the CQ (37%) and SP (32%) groups compared to 22% and 28% in the AQ and AQ+SP groups respectively and this difference was significant p=0.01. Headache was reported more frequently in the CQ (27%) and SP (20%) groups compared to 16% and 15% in the AQ and AQ+SP groups respectively and this difference was significant p=0.01. The frequencies of reports of general weakness, dizziness vomiting, nausea, itching and inhibition of performance of household chores were similar in all the treatment groups ($p<0.1$). Compared to the CQ group, women in the AQ (OR=0.5; 95% CI, 0.3 to 0.7 $p<0.001$) and AQ+SP combination (OR=0.7; 95% CI, 0.4 - 1.0 p=0.05) groups respectively, were less likely to report an adverse

effect; but reports of an adverse effect by women in the SP group (OR=0.8; 95% CI; 0.5 - 1.2 p=0.2) were no more different from the CQ group.

The incidence of skin rash among the study women after the start of treatment is shown in **Table 6.6.**

Table 6.6: - Incidence of skin rash among study women after the start of treatment.

	Incidence of skin rash at follow up days											
	DAY 3			DAY 7			DAY 14			DAY 28		
	N	*n*	*(%)*	*N*	*n*	*(%)*	*N*	*n*	*(%)*	*N*	*n*	*(%)*
Treatment												
CQ	215	5	(2.3)	219	3	(1.4)	216	2	(0.9)	206	4	(1.9)
AQ	220	1	(0.5)	218	3	(1.4)	220	3	(1.4)	215	4	(1.9)
SP	217	3	(1.4)	218	6	(2.8)	215	5	(2.3)	208	3	(1.4)
AQ+SP	217	4	(1.8)	216	4	(1.9)	214	4	(1.9)	213	2	(0.9)
Total	869	13	(1.5)	871	16	(1.8)	865	14	(1.6)	842	13	(1.5)
p-value			**0.4**			**0.7**			**0.7**			**0.8**

As shown in **Table 6.6,** 13 (1.5%) of the study women reported diffuse skin rashes on day 3. The rashes looked coarse and prickly resembling heat rashes and in 11 of the women they were itchy. The highest incidence was in the CQ group followed by the AQ+SP combination group and the SP and AQ groups but there was no statistical difference between the groups. The mucous membranes were not affected nor were there other clinical manifestations of severe cutaneous adverse reactions (SCAR). The women did not need any medical attention for the skin rash. By day 7, only in one of these women did the rash persist but cleared before day 14. The timing of the rashes makes it possible for them to be related to the test drugs, but they could also have occurred by chance or been produced by interactions between the test drugs and concomitant drugs unknown to the investigators.

By day 7, 15 new case of skin rashes similar in appearance to those described above were reported and investigated. But in two of the women, the skin rashes were diffuse maculo-papular non-vesicular rashes, itchy and slightly hyperpigmented. There were no manifestations of SCAR

but the rashes could be related to the test drugs. The pictures of the skin rashes in the two women are shown in **Figures 6.2 and 6.3**

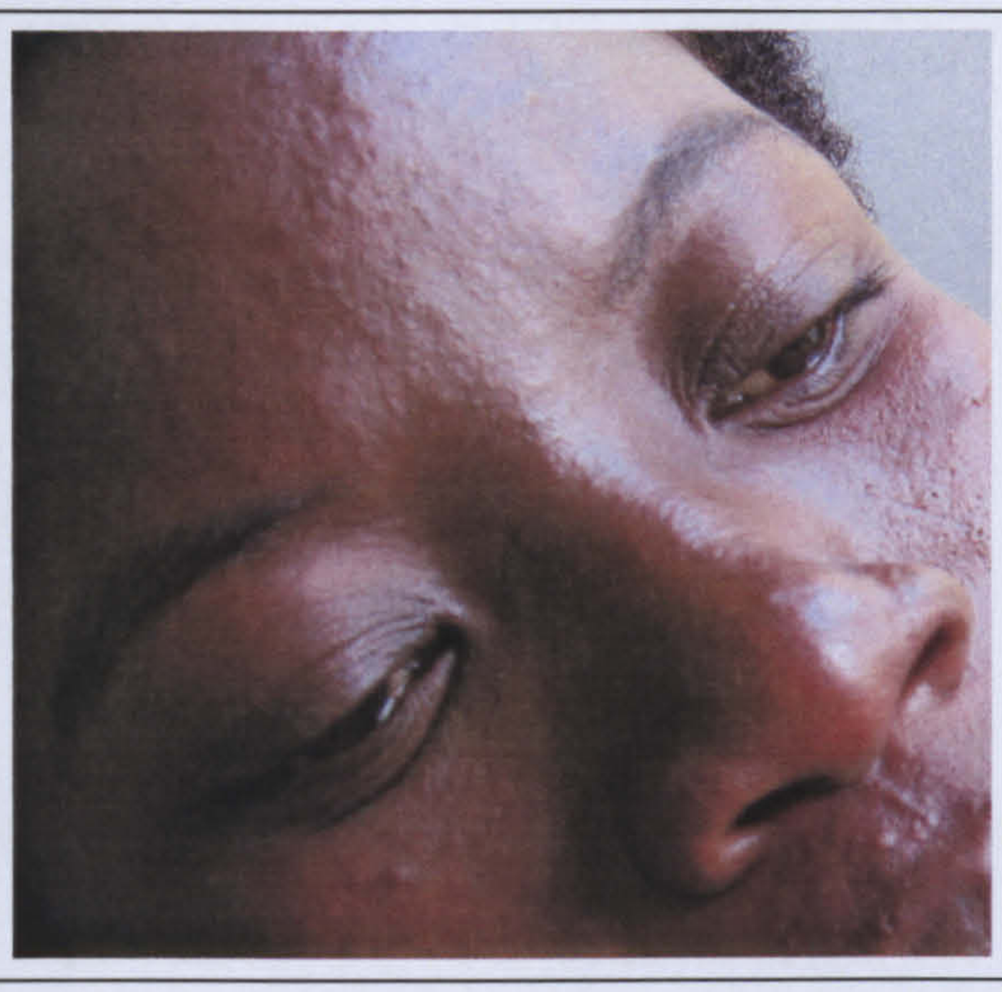


Figure 6.2: - Skin rashes reported at day 7 after treatment with SP.

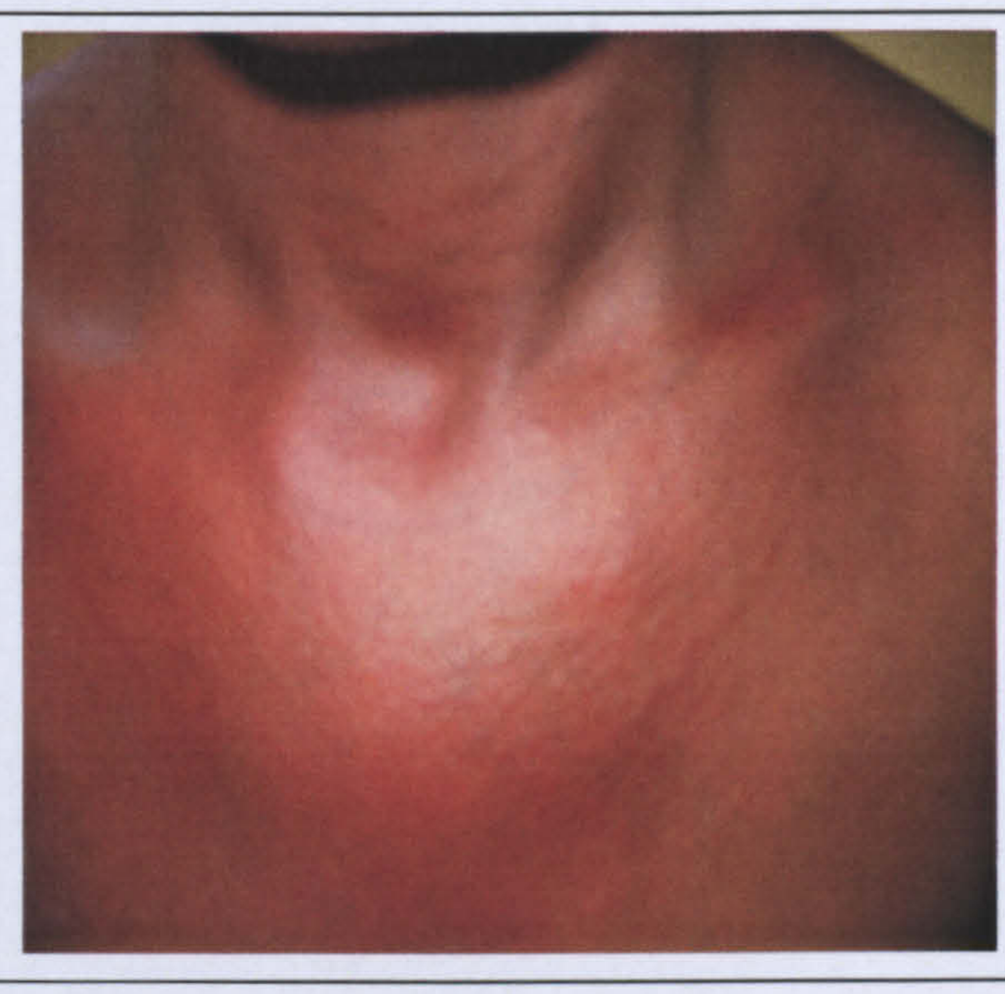


Figure 6.3: - Skin rash reported at day 7 after treatment with AQ+SP.

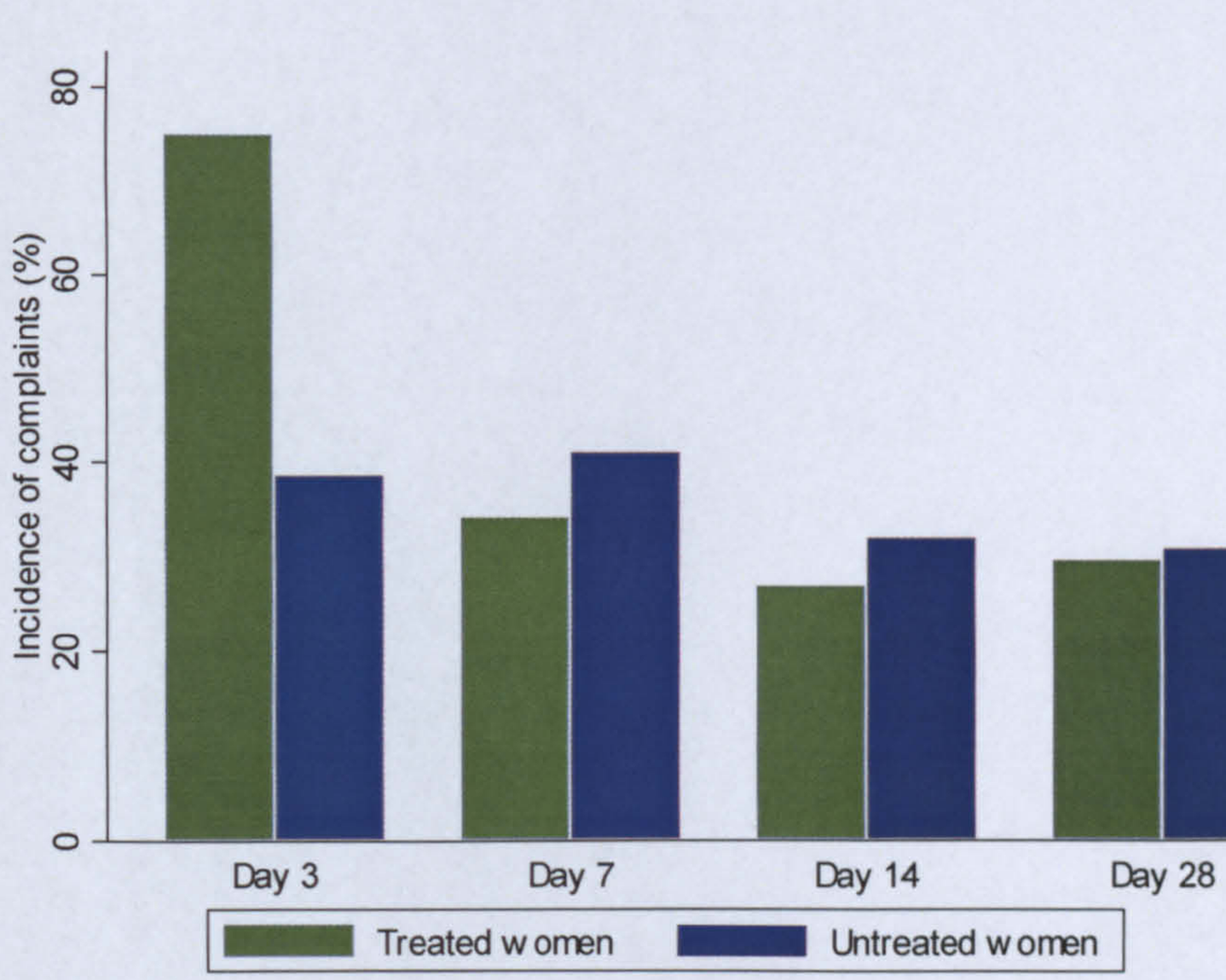
The women did not have any other complaints and did not require any treatment. The rashes disappeared before day 14.

Fourteen and thirteen new reports of skin rashes were received and investigated on days 14 and 28 respectively. In ten of the cases (4 on day 14 and 6 on day 28) fungal skin infections were diagnosed and treated accordingly. There were no manifestations of SCAR in the rest of the cases. In almost all the rest the reported rashes, were more like lichenifications resulting from excessive scratching as they also reported itching. None of these rashes could be associated with the test drugs.

6.2.1 Prevalence of clinical complaints among study population

A comparison between a random sample of pregnant women with negative OptiMAL® antigen tests during screening and the study pregnant women with respect to complaints made by them during 28 days follow up is shown in **Figure 6.4.** On day 3 after treatment, 75% of pregnant women in the study group reported a clinical complaint compared to 39% in the control group. By day 7, the proportion of women with complaints in the study group had reduced to almost half of the day 3 proportions and remained almost stabilized throughout the follow up period. Apart from day 3, the prevalence of clinical complaints in the study group were comparable to those made by pregnant women who were not treated.

Figure 6.4: Comparison of incidence of complaints by treated and untreated pregnant women over a 28-day follow up period after screening.



6.3 Baseline biochemistry measurements

The pre-medication levels of bilirubin, AST, ALT and GGT of the study women in the four treatment groups are shown in **Table 6.7.** There were no significant statistical differences between the groups with respect to these parameters.

Table 6.7: - Baseline biochemistry values according to treatment groups

	CQ		AQ		SP		AQ+SP		Total	
	n	*(%)*	*n*	*(%)*	*n*	*(%)*	*n*	*(%)*	*n*	*(%)*
AST										
Up to 12 U/L	129	(59.5)	127	(58.5)	124	(57.7)	134	(60.4)	514	(59)
13 to 24 U/L	61	(28.1)	65	(29.9)	69	(32.1)	67	(30.2)	262	(30.1)
=/> 25 U/L	27	(12.4)	25	(11.5)	22	(10.2)	21	(9.5)	95	(10.9)
Mean (SD)	13.9	(10.6)	14.2	(11.6)	14.0	(11)	13.4	(10.1)	13.9	(10.8)
Median (IQR)	10	(9)	10	(9)	10	(9)	10	(9)	10	(9)
ALT										
Up to 12 U/L	184	(85.2)	184	(84.8)	188	(87.4)	199	(89.6)	755	(86.8)
13 to 24 U/L	23	(10.6)	19	(8.8)	17	(7.9)	18	(8.1)	77	(8.8)
=/> 25 U/L	9	(4.2)	14	(6.4)	10	(4.7)	5	(2.3)	38	(4.4)
Mean (SD)	7.5	(6.6)	7.8	(8.3)	7.8	(8.6)	7.0	(7.4)	7.5	(7.8)
Median (IQR)	4	(4)	4	(4)	4	(4)	4	(4)	4	(4)
GGT										
Up to 50 U/L	212	(99.5)	213	(99.1)	212	(99.5)	218	(100)	855	(99.5)
51 to 100 U/L	1	(0.5)	2	(0.9)	0	(0)	0	(0)	3	(0.4)
=/> 101 U/L	0	(0)	0	(0)	1	(0.5)	0	(0)	1	(0.1)
Mean (SD)	9.4	(8)	10.4	(10.2)	10.4	(11.6)	9.3	(6.6)	9.9	(9.3)
Median (IQR)	7	(6)	8	(7)	8	(7)	8	(7)	8	(7)
Total Bilirubin (µmol/L)										
Up to 17.1 µmol/L	176	(81.1)	173	(79.7)	171	(79.5)	193	(86.9)	713	(81.9)
17.2 to 34.4 µmol/L	29	(13.4)	34	(15.7)	31	(14.4)	23	(10.4)	117	(13.4)
=/> 34.5 µmol/L	12	(5.5)	10	(4.6)	13	(6.1)	6	(2.7)	41	(4.7)
Mean (SD)	12.6	(17.6)	12.3	(11.5)	12.6	(12.4)	11.0	(11)	12.1	(13.4)
Median (IQR)	8	(9)	9	(10)	8	(10)	7	8	8	(9)
Direct Bilirubin (µmol/L)										
Up to 3.4 µmol/L	119	(54.8)	119	(54.8)	127	(59.1)	141	(63.5)	506	(58.1)
3.5 to 6.8 µmol/L	51	(23.5)	57	(26.3)	46	(21.4)	51	(23)	205	(23.5)
=/> 6.9 µmol/L	47	(21.7)	41	(18.9)	42	(19.5)	30	(13.5)	160	(18.4)
Mean (SD)	5.2	(6.6)	5.0	(5.6)	5.0	(5.8)	4.3	(5.5)	4.9	(5.9)
Median (IQR)	3	(4)	3	(4)	3	(4)	2.6	(3)	3	(4)
Indirect Bilirubin (µmol/L)										
Up to 13.7µmol/L	192	(88.5)	191	(88)	187	(87)	205	(92.3)	775	(89)
13.8 to 27.4 µmol/L	22	(10.1)	20	(9.2)	21	(9.8)	14	(6.3)	77	(8.8)
=/> 27.5 µmol/L	3	(1.4)	6	(2.8)	7	(3.3)	3	(1.4)	19	(2.2)
Mean (SD)	7.4	(11.9)	7.3	(7.3)	7.6	(8)	6.6	(6.9)	7.2	(8.7)
Median (IQR)	5	(5)	5	(6)	5	(6)	5	(5)	5	(6)

As shown in **Table 6.7**, apart from AST and direct bilirubin, all the other parameters had their baseline mean values within normal ranges. At baseline, 59% of the women had AST levels within the normal range, 30% were above the normal range but within 2 times the upper limit and 11% had values 2 times above the upper limit of the normal range. The overall mean of AST level was 13.9 U/L at enrolment. Eighty-seven percent of women had ALT levels within the normal range, 9% were above the normal range but within 2 times the upper limit and 4% were 2 times above the upper limit of the normal range. The overall baseline mean of ALT level was 9.9 U/L. At baseline, 99.5% of the women had GGT levels within the normal range with an overall mean level of 9.9 U/L. Eighty-two percent of the women had total bilirubin levels within the normal range, 13% were above the normal range but within 2 times the upper limit and 5% were 2 times above the upper limit of the normal range. The overall baseline mean of total bilirubin level was 12.1μmol/L. Fifty-eight percent of the women had direct bilirubin levels within the normal range, 24% were above the normal range but within 2 times the upper limit and 18% were 2 times above the upper limit of the normal range. The overall baseline mean of direct bilirubin level was 4.9μmol/L. Urea and creatinine levels were determined on a subset of samples only. Ninety-five percent and 76% of the women respectively had urea and creatinine levels within normal range at enrolment.

6.4 Post treatment biochemistry measurements

6.4.1 Aspartate aminotransferase (AST)

The distribution of pregnant women according to treatment groups within predefined levels of AST at days 14 and 28 after the start of treatment are shown in **Table 6.8 and Figure 6.5** respectively.

Table 6.8: AST levels at days 14 and 28 after treatment according to treatment group.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DAY 14										
Up to 12 U/L	86	(43.2)	99	(49)	90	(43.9)	98	(49)	373	(46.3)
13 to 24 U/L	86	(43.2)	70	(34.7)	85	(41.5)	75	(37.5)	316	(39.2)
=/> 25 U/L	27	(13.6)	33	(16.3)	30	(14.6)	27	(13.5)	117	(14.5)
Mean (SD)	15.3	(9.8)	17.5	(15.7)	16.2	(12.7)	16.0	(13)	16.2	(13)
Median (IQR)	13	(12)	13	(12)	13	(12)	13	(12)	13	(12)
DAY 28										
Up to 12 U/L	100	(52.9)	114	(54)	106	(54.6)	115	(58.4)	435	(55)
13 to 24 U/L	63	(33.3)	82	(38.9)	69	(35.6)	66	(33.5)	280	(35.4)
=/> 25 U/L	26	(13.8)	15	(7.1)	19	(9.8)	16	(8.1)	76	(9.6)
Mean (SD)	15.1	(12.5)	13.2	(9.4)	14.3	(12)	13.2	(10.5)	13.9	(11.1)
Median (IQR)	10.0	(11)	10.0	(9)	10.0	(12)	10.0	(9)	10.0	(9)

As shown in **Table 6.8** and **Figure 6.6**, there was a general increase in AST levels within treatment groups over the follow up period after treatment. The proportions of women within the normal range decreased to 46% on day 14 and then increased again to 55% at day 28 compared to 59% at enrolment. The pattern was the same across the treatment groups and a comparison of proportions did not show any significant statistical differences between them.

Also within all the treatment groups mean AST levels increased at day 14 but decreased to baseline means at day 28 as shown in **Figure 6.6.**

Figure 6.5: - Levels of AST at enrolment and days 14 and 28 according to treatment groups

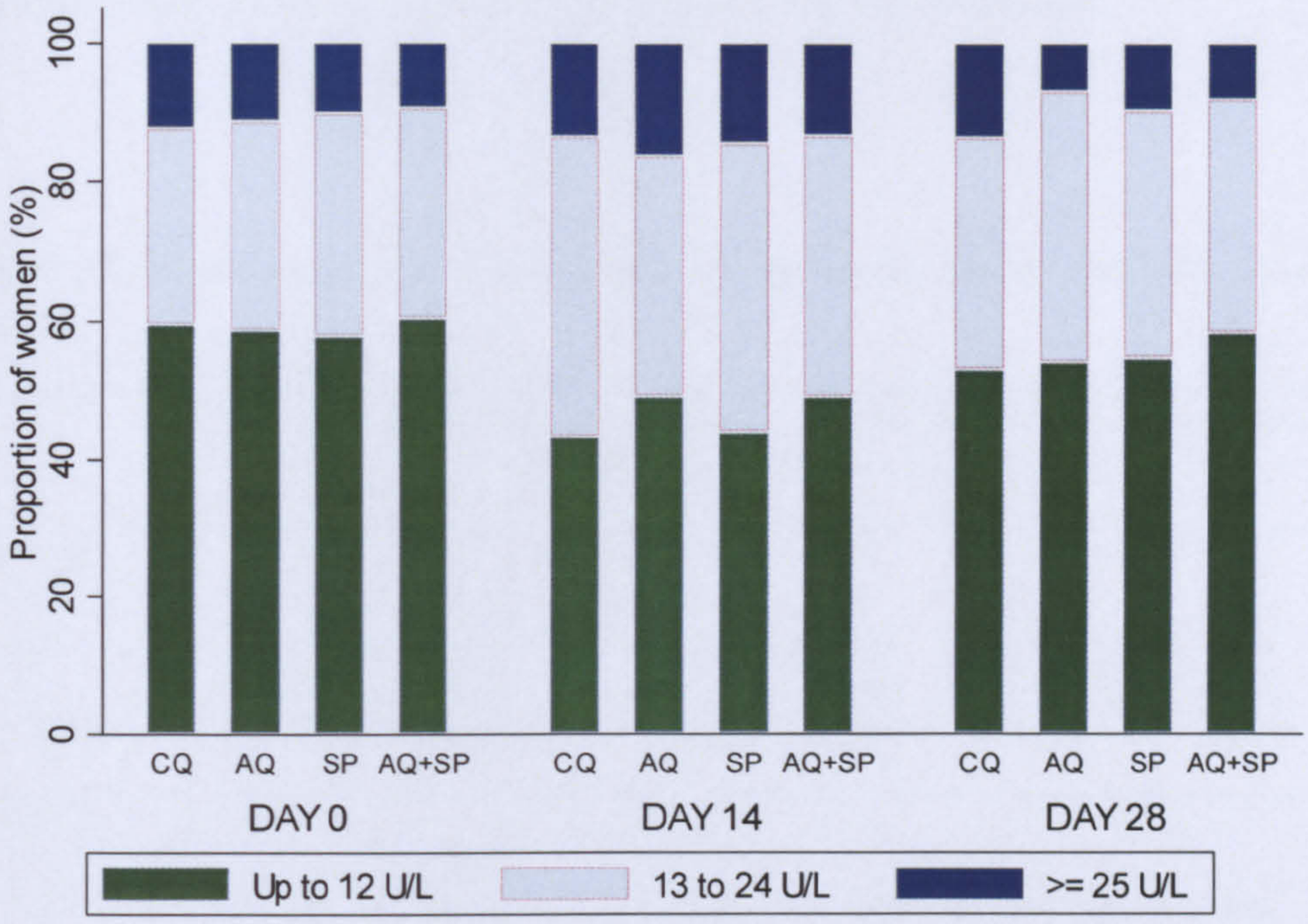
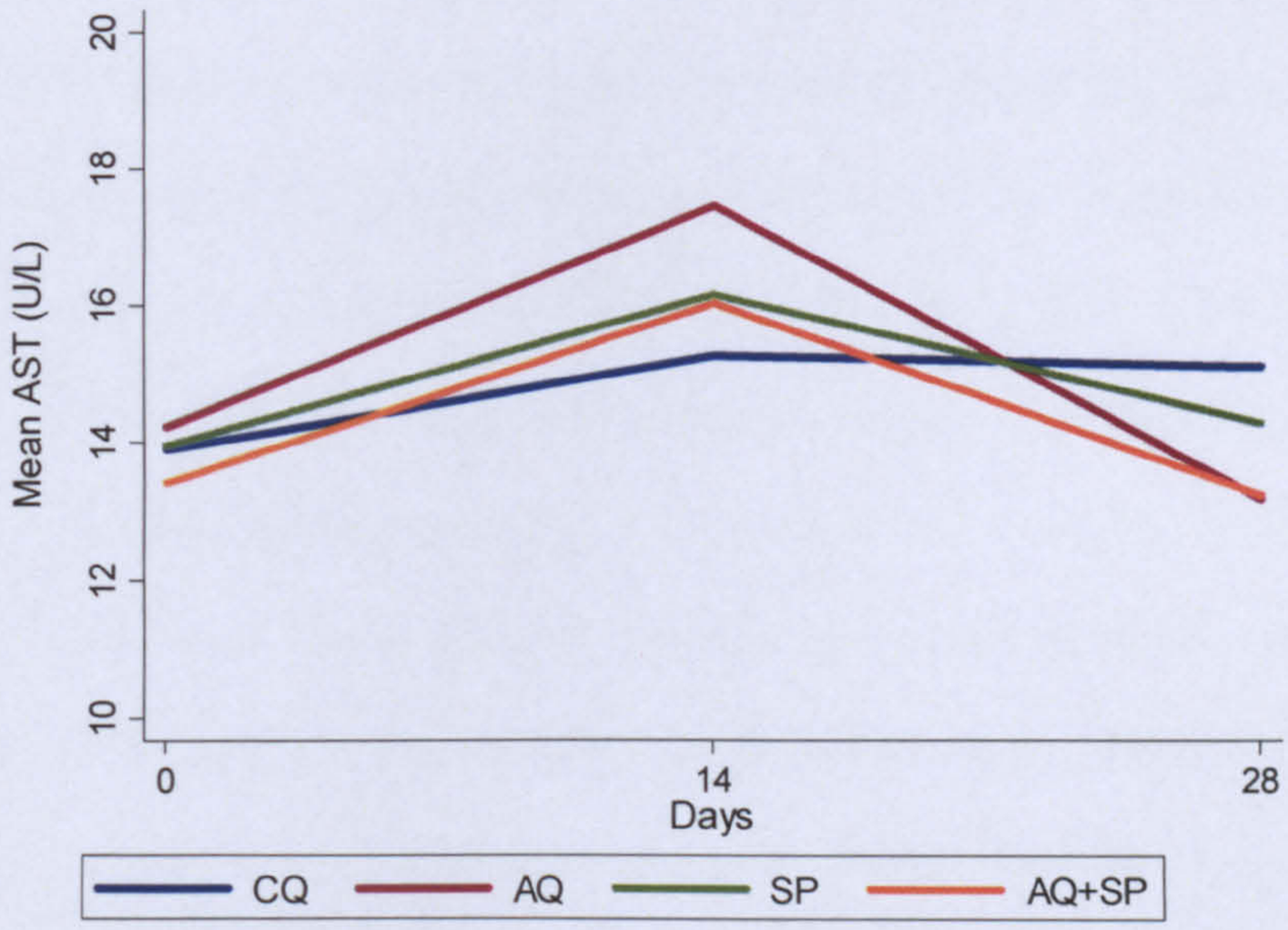


Figure 6.6: - Mean AST levels at enrolment and at days 14 and 28 after start of treatment according to treatment group.



The mean changes in AST activity at days 14 and 28 after the start of treatment relative to baseline values according to treatment groups are shown in **Table 6.9.**

Table 6.9: - Mean changes in AST activity at days 14 and 28 after treatment according to treatment group.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p-value)
DAY 14							
CQ	1.5	(10.4)	(-34 to 38)				0.4(0.8)
AQ	2.9	(16.8)	(-51 to 82)	1.4	(-1.4 to 4.2)	0.32	
SP	2.4	(14.9)	(-48 to 82)	0.9	(-1.9 to 3.7)	0.52	
AQ+SP	2.7	(13.4)	(-39 to 82)	1.2	(-1.6 to 4)	0.40	
DAY 28							
CQ	1.6	(13.3)	(-42 to 73)				1.6(0.2)
AQ	-1.3	(11.9)	(-51 to 61)	-2.9	(-5.5 to -0.2)	0.04	
SP	0.8	(13.7)	(-57 to 82)	-0.8	(-3.5 to 1.9)	0.56	
AQ+SP	0.2	(14.4)	(-45 to 79)	-1.3	(-4 to 1.3)	0.33	

There was an overall mean increase of 2.4 U/L (95% CI; 1.4 to 3.4 $p<0.001$) in AST activity at day 14 relative to baseline mean values. As shown in **Table 6.9,** there is no association between this increase and the type of treatment received (F-statistic = 0.4; $p=0.8$). A comparison of the test drugs with CQ showed that the mean AST change was 1.4, 0.9 and 1.2 U/L more in the AQ, SP and AQ+SP groups respectively compared to the CQ group but these differences are not statistically significant. At day 28, the overall mean increase in AST activity was 0.3 U/L (95% CI; -0.6 to 1.2 $p=0.6$) over baseline values and showed no association with treatment received (F-statistic = 1.6 and $p=0.2$). The mean changes were about 2.9, 0.8 and 1.3 units respectively less in the AQ, SP and AQ+SP groups compare to the CQ group. The differences were statistically not significant for the CQ versus SP and AQ+SP comparisons but significant in the case of CQ versus AQ (p – value =0.04). Overall the differences between the groups were

not statistically significant. The mean changes in AST activity remained unchanged when baseline parasite density, parity or gravidity were included in the regression model.

6.4.2 Alanine aminotransferase (ALT)

The distribution of pregnant women according to treatment groups within predefined levels of ALT at days 14 and 28 after the start of treatment are shown in **Table 6.10 and Figure 6.7** respectively.

Table 6.10: - ALT levels at days 14 and 28 after treatment according to treatment group.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DAY 14										
Up to 12 U/L	175	(88)	171	(84.7)	175	(85.4)	176	(88)	697	(86.5)
13 to 24 U/L	12	(6)	25	(12.4)	19	(9.3)	16	(8)	72	(8.9)
=/> 25 U/L	12	(6)	6	(2.9)	11	(5.3)	8	(4)	37	(4.6)
Mean (SD)	8.0	(8.3)	7.5	(6.8)	8.2	(8.8)	7.7	(8.5)	7.9	(8.1)
Median (IQR)	4	(4)	4	(4)	4	(4)	4	(4)	4	(4)
DAY 28										
Up to 12 U/L	167	(88.4)	186	(88.2)	171	(88.1)	177	(89.9)	701	(88.6)
13 to 24 U/L	17	(9)	23	(10.9)	18	(9.3)	15	(7.6)	73	(9.2)
=/> 25 U/L	5	(2.6)	2	(0.9)	5	(2.6)	5	(2.5)	17	(2.2)
Mean (SD)	7.2	(5.6)	6.7	(5)	7.0	(5.6)	6.7	(6.4)	6.9	(5.6)
Median (IQR)	4.0	(4)	4.0	(4)	4.0	(4)	4.0	(4)	4.0	(4)

As shown in **Table 6.10**and **Figure 6.8**, the overall proportion of women with a normal range of ALT activity was similar at enrolment and on days 14 and 28 after treatment. The mean ALT levels increased at day 14 for CQ, SP and AQ+SP but decreased to baseline means at day 28 as shown in **Figure 7.8**. In the AQ group the mean ALT activity decreased at the follow up times compared to enrolments.

Figure 6.7: - Levels of ALT activity at enrolment and days 14 and 28 according to treatment groups

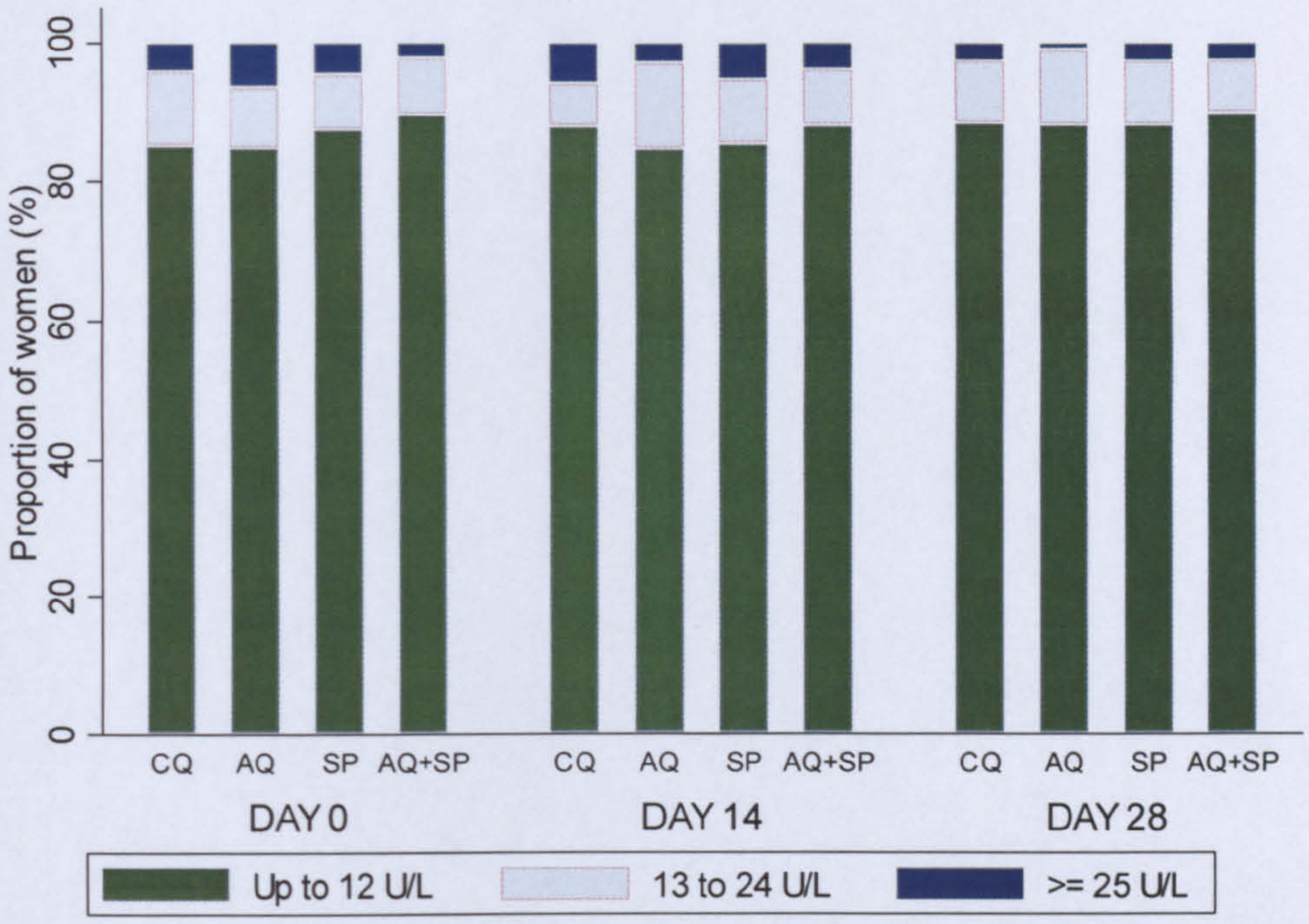
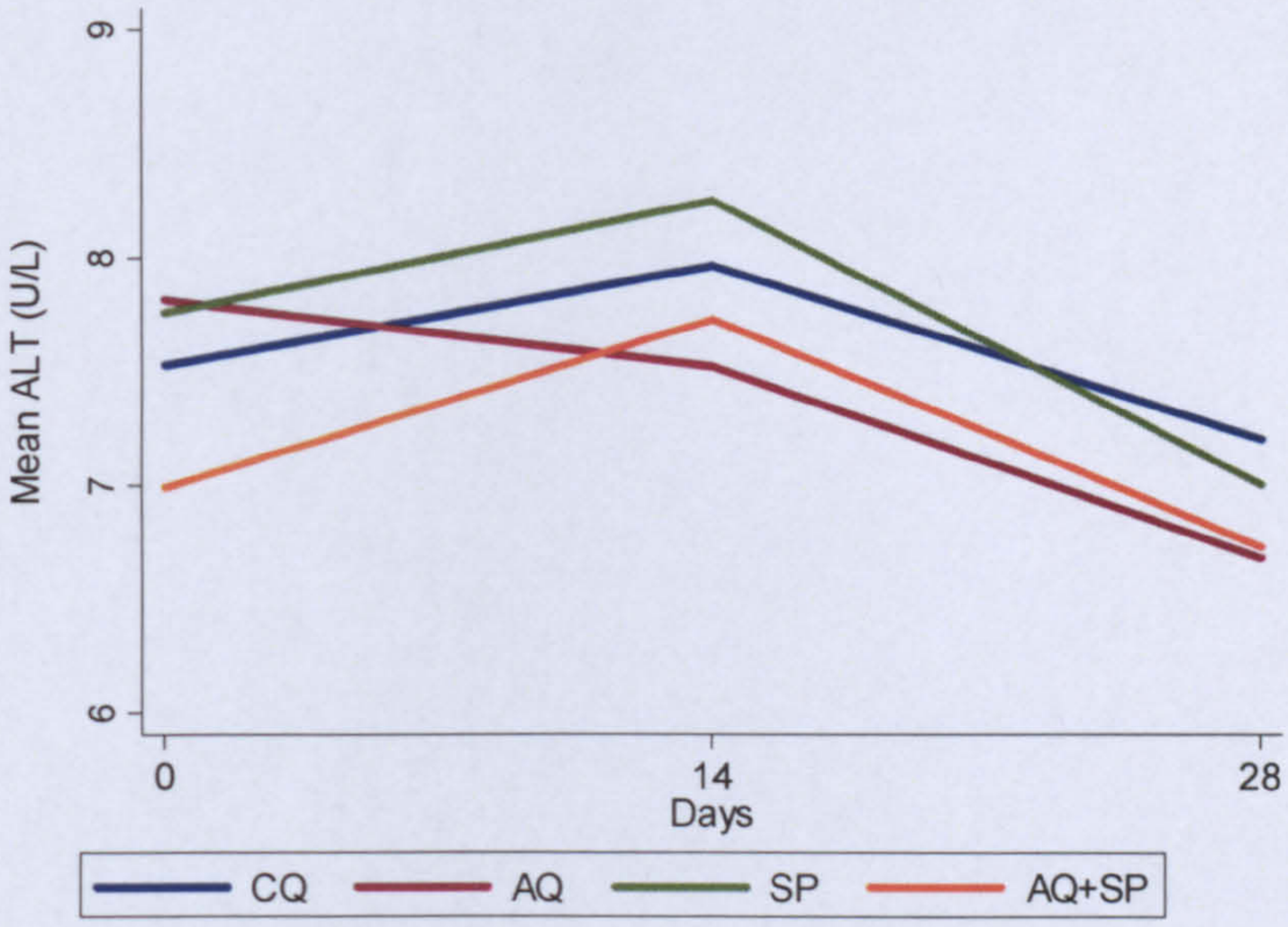


Figure 6.8: - Mean ALT levels at enrolment and at days 14 and 28 after start of treatment according to treatment group.



The mean changes in ALT activity at days 14 and 28 after the start of treatment relative to baseline values according to treatment groups are shown in **Table 6.11.**

Table 6.11: - Mean changes in ALT activity at days 14 and 28 after treatment according to treatment group.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p-value)
DAY 14							
CQ	0.5	(8)	(-24 to 44)				0.3(0.9)
AQ	0.0	(8.1)	(-53 to 28)	-0.5	(-2.3 to 1.3)	0.57	
SP	0.6	(9.8)	(-45 to 59)	0.1	(-1.7 to 1.9)	0.88	
AQ+SP	0.7	(9.8)	(-61 to 43)	0.2	(-1.6 to 2)	0.81	
DAY 28							
CQ	-0.2	(7.1)	(-26 to 25)				0.8(0.5)
AQ	-1.3	(7.2)	(-52 to 13)	-1.0	(-2.7 to 0.6)	0.21	
SP	-0.6	(9.1)	(-49 to 29)	-0.4	(-2.2 to 1.3)	0.64	
AQ+SP	-0.1	(9.1)	(-67 to 54)	0.1	(-1.5 to 1.8)	0.87	

Overall, there was no significant difference between mean ALT activity at day 14 and at enrolment but a difference of borderline significance ($p = 0.06$) was noted at day 28.

6.4.3 Gamma-glutamyl transferase (GGT)

The distribution of pregnant women according to treatment groups within predefined levels of GGT at days 14 and 28 after the start of treatment are shown in **Table 6.12 and Figure 6.9** respectively.

Table 6.12: - GGT levels at days 14 and 28 after treatment according to treatment group.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DAY 14										
Up to 50 U/L	197	(92)	201	(94.4)	203	(93.6)	199	(92.6)	800	(93.1)
51 to 100 U/L	1	(0.5)	0	(0)	2	(0.9)	1	(0.5)	4	(0.5)
=/> 101 U/L	16	(7.5)	12	(5.6)	12	(5.5)	15	(6.9)	55	(6.4)
Mean (SD)	15.9	(25)	14.8	(22)	15.4	(22.8)	15.7	(24.6)	15.4	(23.6)
Median (IQR)	8	(8)	9	(8)	8	(8)	8	(9)	8	(8)
DAY 28										
Up to 50 U/L	188	(86.6)	210	(94.6)	192	(89.3)	194	(88.6)	784	(89.8)
51 to 100 U/L	0	(0)	1	(0.5)	1	(0.5)	2	(0.9)	4	(0.5)
=/> 101 U/L	29	(13.4)	11	(4.9)	22	(10.2)	23	(10.5)	85	(9.7)
Mean (SD)	21.6	(32)	13.9	(21.5)	18.6	(28.6)	19.0	(29.2)	18.2	(28.1)
Median (IQR)	9.0	(10)	8.0	(7)	9.0	(7)	8.0	(8)	8.0	(8)

Overall, the proportion of women with GGT activity outside the normal range increased on day 14 with a further increase on day 28 as shown in **Figures 6.9.**

Figure 6.9: - Levels of GGT activity at enrolment and days 14 and 28 according to treatment groups

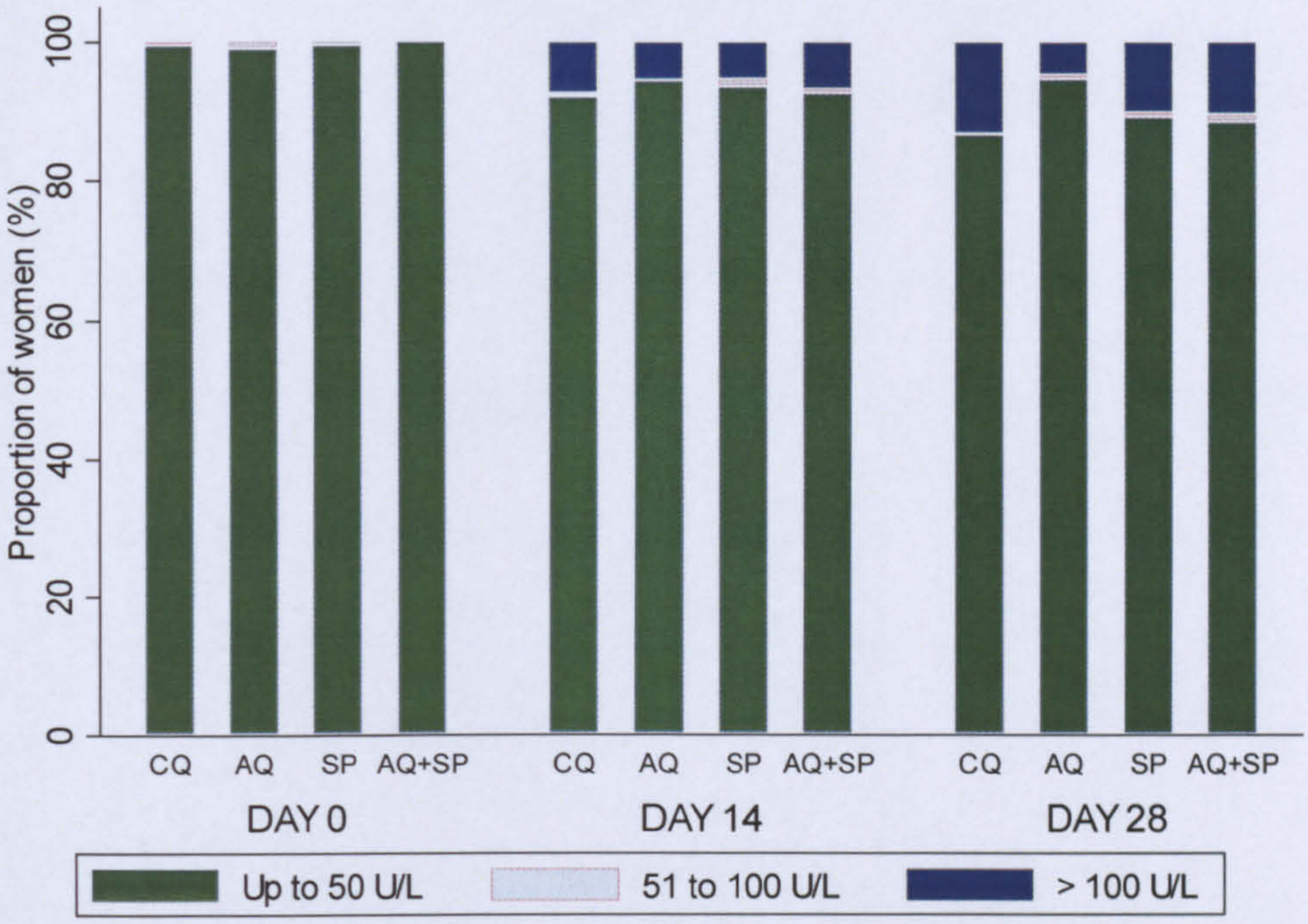
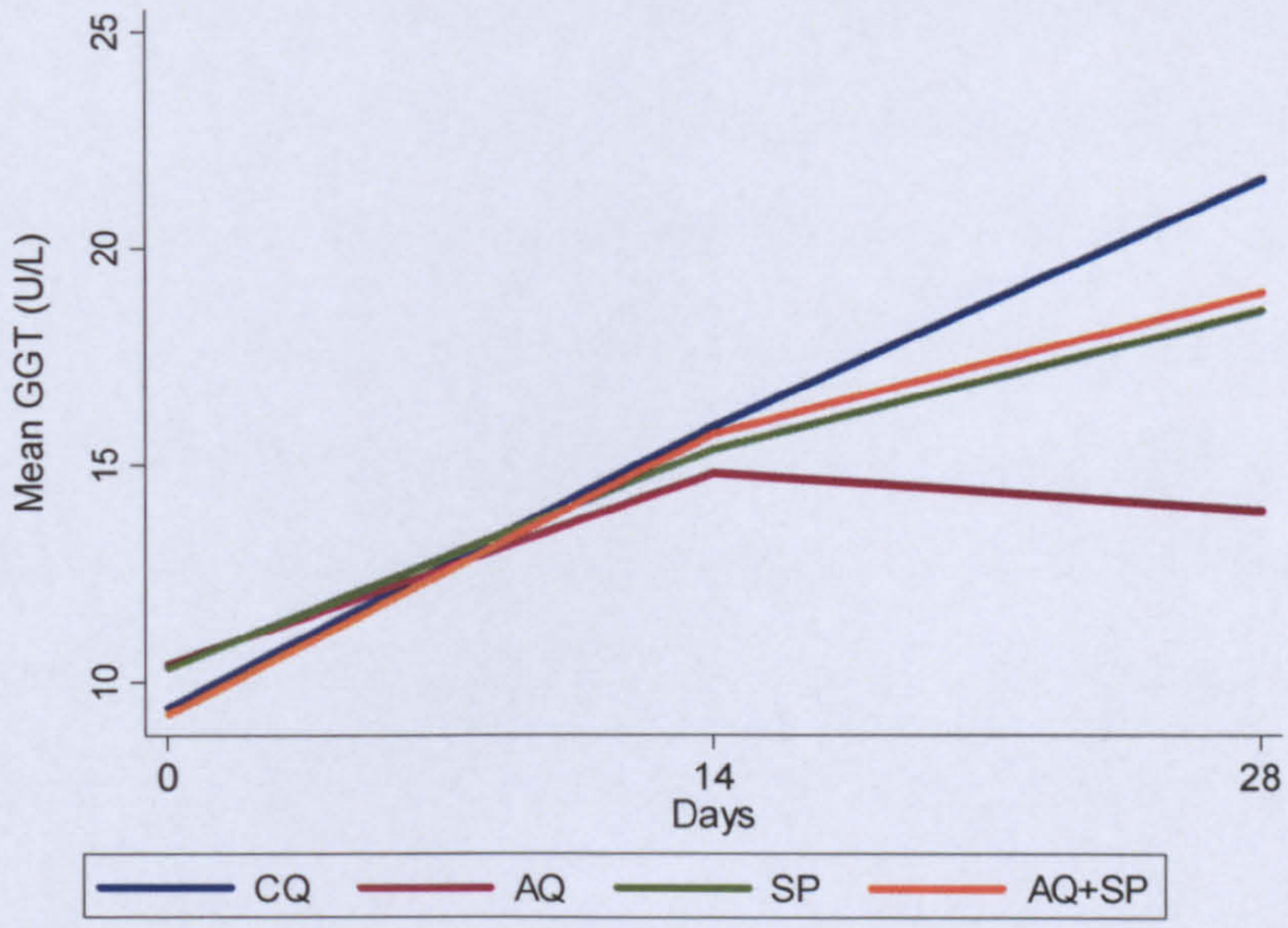


Figure 6.10: - Mean GGT levels at enrolment and at days 14 and 28 after start of treatment according to treatment group.



The mean changes in GGT activity at days 14 and 28 after the start of treatment relative to baseline values according to treatment groups are shown in **Table 6.13**.

Table 6.13: - Mean changes in GGT activity at days 14 and 28 after treatment according to treatment group.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p-value)
DAY 14							
CQ	5.6	(24.1)	(-44 to 99)				0.3(0.8)
AQ	4.8	(22.4)	(-38 to 98)	-0.8	(-5.4 to 3.8)	0.74	
SP	4.8	(22.8)	(-59 to 97)	-0.8	(-5.4 to 3.8)	0.73	
AQ+SP	6.7	(25.4)	(-44 to 98)	1.1	(-3.5 to 5.7)	0.64	
DAY 28							
CQ	11.5	(31.4)	(-42 to 98)				2.8(0.04)
AQ	4.0	(22.2)	(-53 to 95)	-7.5	(-12.9 to -2.1)	0.01	
SP	8.3	(28.5)	(-39 to 99)	-3.2	(-8.7 to 2.2)	0.25	
AQ+SP	10.0	(29.7)	(-46 to 98)	-1.5	(-6.9 to 3.9)	0.59	

The overall mean increase in GGT activity at day 14 was 5.5 U/L (95% CI; 3.8 to 7.1 $p<0.001$) over the baseline mean but this increase was not related to the type of treatment (F-statistic = 0.3 and $p=0.8$). At day 28, the overall mean increase in GGT activity was about 8.4 U/L (95% CI; 6.5 to 10.4 $p<0.001$) over the baseline mean and the increase was associated with the type of treatment (F-statistic = 2.8 and $p=0.04$). The increase was significantly less in the AQ group ($p=0.01$). The mean increase in the CQ group was 7.5, 3.2 and 1.5 U/L more than in the AQ, SP and AQ+SP groups respectively as shown in **Table 6.13**. The mean changes in GGT activity remained unchanged when baseline parasite density, parity or gravidity were included in the regression model.

6.4.4 Overall bilirubin concentrations

The distribution of pregnant women according to treatment groups within predefined levels of bilirubin concentrations at days 14 and 28 after the start of treatment are shown in **Tables 6.14, 6.15 and 6.16 and Figures 6.11, 6.12 and 6.13** respectively.

The proportion of women within the normal range for overall bilirubin concentration increased over enrolment at days 14 and 28 after the start of treatment. Eighty-two percent of the women had normal total bilirubin levels at enrolment; this increased to 91% at day 14 and 92% at day 28. The proportion of women with a normal direct bilirubin increased from 58% at enrolment to 77% at day 14 and 75% at day 28. The proportion with a normal indirect bilirubin also increased from 89% at enrolment to 97% at day 14 and day 28. This pattern was similar in all the treatment groups over the follow up periods as illustrated by **Figures 6.11, 6.12 and 6.13** and did not differ significantly between treatment groups.

Table 6.14: - Total bilirubin levels at days 14 and 28 after treatment according to treatment group.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DAY 14										
Up to 17.1 µmol/L	178	(89.5)	180	(89.1)	191	(93.2)	185	(92.5)	734	(91.1)
17.2 to 34.4 µmol/L	14	(7)	21	(10.4)	11	(5.4)	9	(4.5)	55	(6.8)
=/> 34.5 µmol/L	7	(3.5)	1	(0.5)	3	(1.4)	6	(3)	17	(2.1)
Mean (SD)	9.4	(10.9)	8.6	(7.1)	7.5	(6.4)	8.2	(7.8)	8.4	(8.2)
Median (IQR)	6	(5)	6	(6)	5	(4)	5	(4)	6	(5)
DAY 28										
Up to 17.1 µmol/L	175	(92.6)	192	(91)	175	(90.2)	183	(92.9)	725	(91.7)
17.2 to 34.4 µmol/L	9	(4.8)	16	(7.6)	14	(7.2)	10	(5.1)	49	(6.2)
=/> 34.5 µmol/L	5	(2.6)	3	(1.4)	5	(2.6)	4	(2)	17	(2.1)
Mean (SD)	8.2	(7)	8.3	(7.3)	8.6	(7.9)	8.1	(6.9)	8.3	(7.3)
Median (IQR)	6.0	(5)	6.0	(5)	6.0	(5)	6.0	(5)	6.0	(5)

Figure 6.11: - Total bilirubin levels at enrolment and days 14 and 28 according to treatment groups

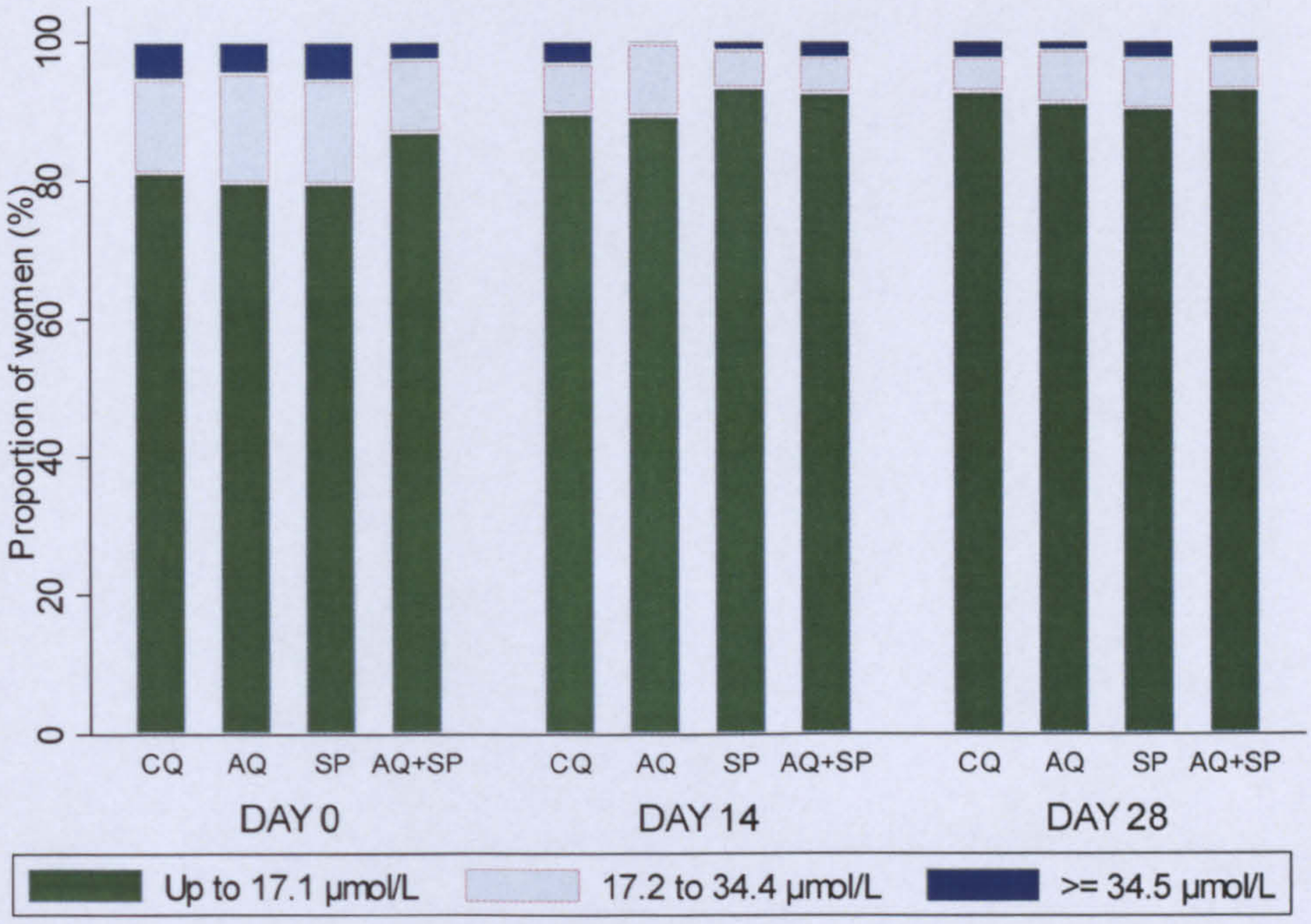


Table 6.15: - Direct bilirubin levels at days 14 and 28 after treatment according to treatment group.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DAY 14										
Up to 3.4 µmol/L	147	(73.9)	152	(75.2)	159	(77.6)	161	(80.5)	619	(76.8)
3.5 to 6.8 µmol/L	26	(13.1)	27	(13.4)	27	(13.2)	17	(8.5)	97	(12)
=/> 6.9 µmol/L	26	(13)	23	(11.4)	19	(9.3)	22	(11)	90	(11.2)
Mean (SD)	3.7	(4.9)	3.4	(3.6)	3.2	(3.6)	3.3	(4.2)	3.4	(4.1)
Median (IQR)	2	(2)	2	(1.1)	2	(2)	2	(1.5)	2	(1)
DAY 28										
Up to 3.4 µmol/L	137	(72.5)	158	(74.9)	146	(75.3)	153	(77.7)	594	(75.1)
3.5 to 6.8 µmol/L	33	(17.5)	31	(14.7)	29	(14.9)	24	(12.2)	117	(14.8)
=/> 6.9 µmol/L	19	(10)	22	(10.4)	19	(9.8)	20	(10.1)	80	(10.1)
Mean (SD)	3.6	(4)	3.4	(3.8)	3.3	(4.1)	3.2	(3.8)	3.4	(3.9)
Median (IQR)	2.0	(2)	2.0	(3)	2.0	(1)	2.0	(1)	2.0	(1.2)

Figure 6.12: - Direct bilirubin levels at enrolment and days 14 and 28 according to treatment groups

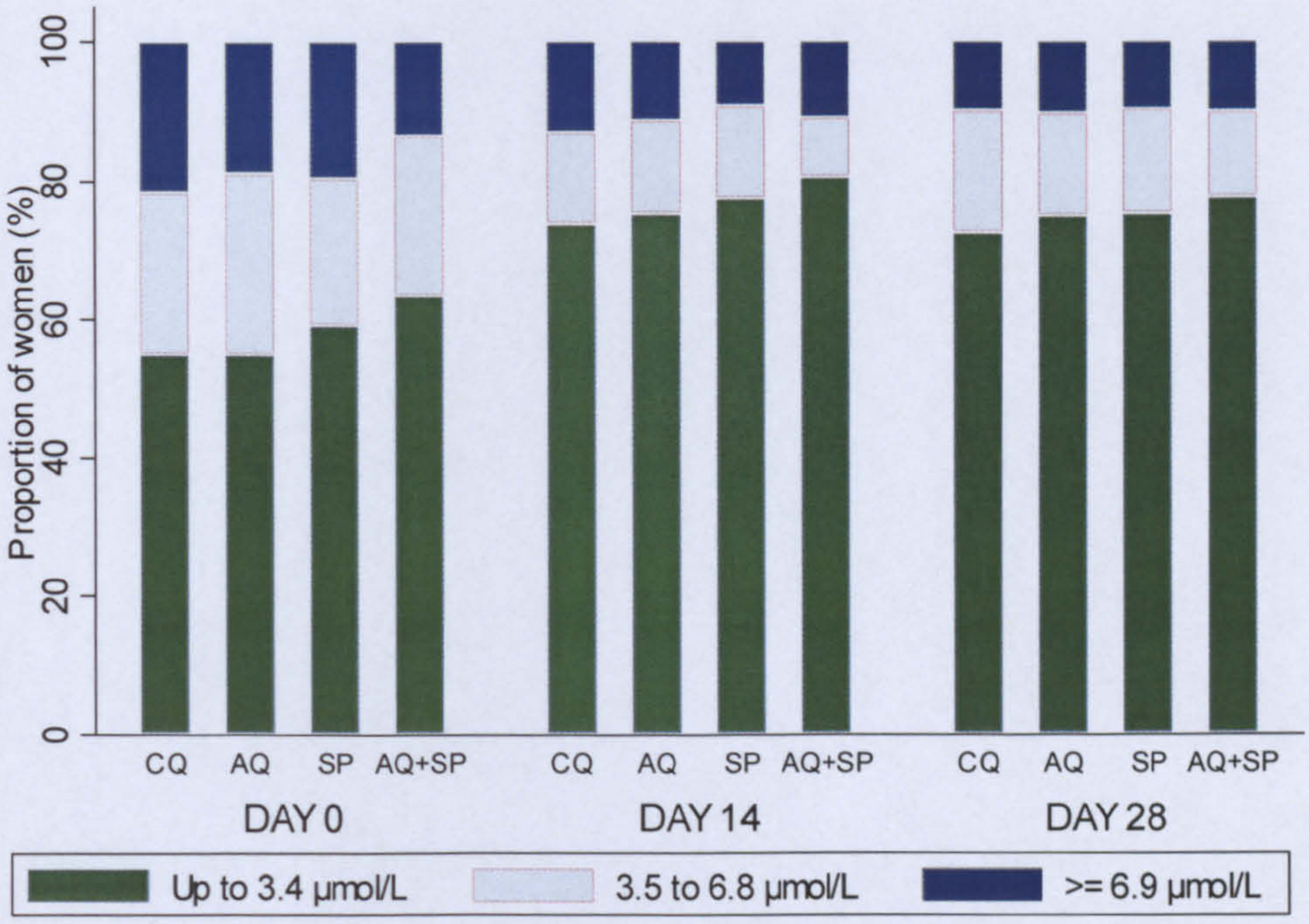
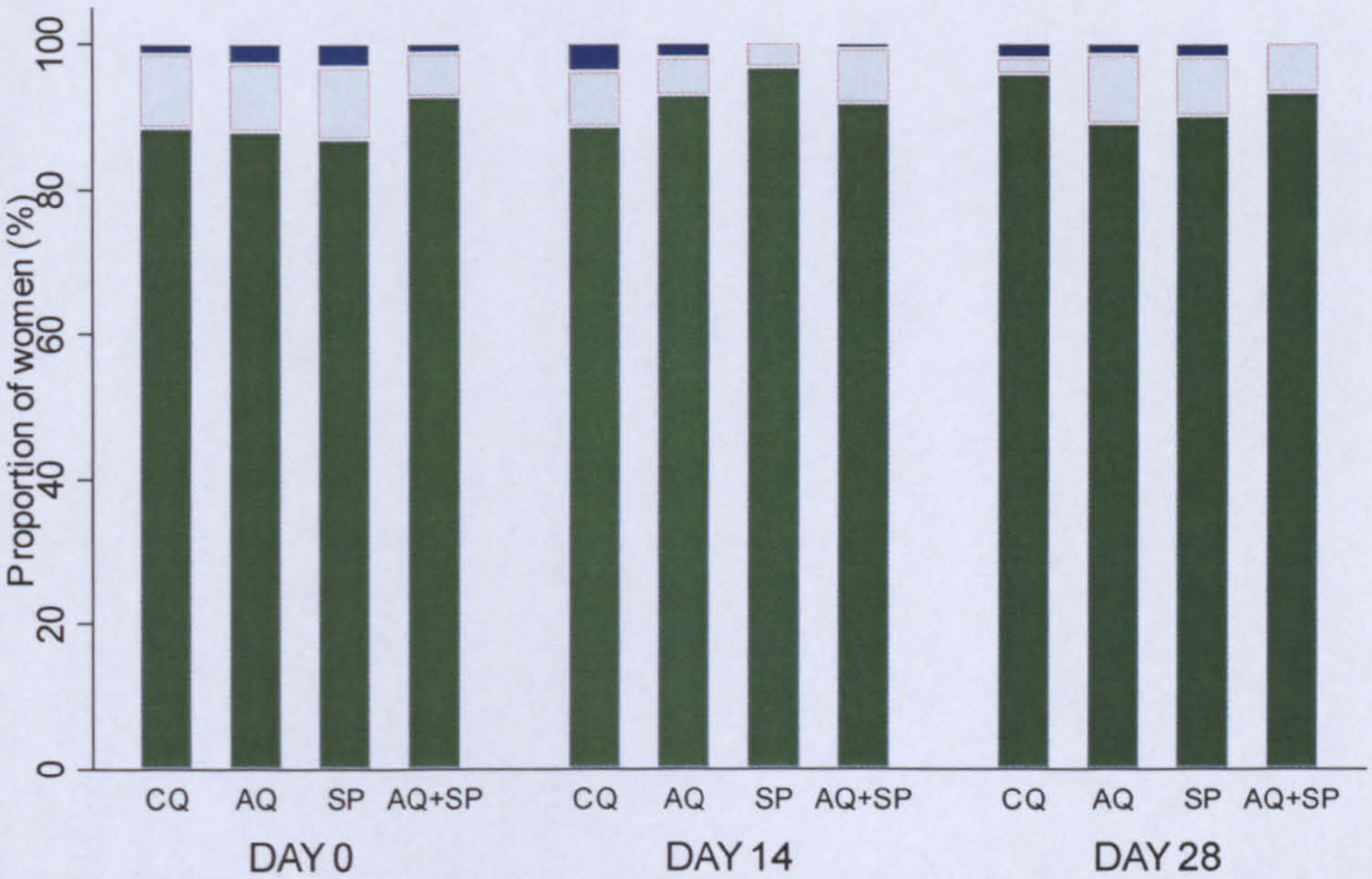


Table 6.16: - Indirect bilirubin levels at enrolment and days 14 and 28 according to treatment groups

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DAY 14										
Up to 13.7μmol/L	191	(96)	196	(97)	200	(97.6)	194	(97)	781	(96.9)
13.8 to 27.4 μmol/L	7	(3.5)	6	(3)	4	(1.9)	5	(2.5)	22	(2.7)
=/> 27.5 μmol/L	1	(0.5)	0	(0)	1	(0.5)	1	(0.5)	3	(0.4)
Mean (SD)	5.7	(6.8)	5.4	(5.3)	4.3	(3.4)	4.8	(4.6)	5.1	(5.2)
Median (IQR)	4	(4)	4	(5)	3	(3)	3	(4)	3	(4)
DAY 28										
Up to 13.7μmol/L	181	(95.8)	204	(96.7)	190	(98)	190	(96.5)	765	(96.7)
13.8 to 27.4 μmol/L	7	(3.7)	7	(3.3)	2	(1)	6	(3)	22	(2.8)
=/> 27.5 μmol/L	1	(0.5)	0	0	2	(1)	1	(0.5)	4	(0.5)
Mean (SD)	4.6	(3.9)	5.0	(4.4)	5.2	(5)	4.9	(3.8)	4.9	(4.3)
Median (IQR)	4.0	(4)	3.7	(4)	4.0	(4)	4.0	(4)	4.0	(4)

Figure 6.13: - Indirect bilirubin levels at enrolment and days 14 and 28 according to treatment groups



The overall mean changes in bilirubin concentrations at days 14 and 28 after the start of treatment relative to baseline values according to treatment groups are shown in **Tables 6.17, 6.18 and 6.19 and Figures 6.14, 6.15 and 6.16**.

The overall mean decrease in total bilirubin level at day 14 was 3.6 μmol/L (95% CI; 2.7 to 4.6 $p<0.001$) over the baseline mean but was not associated with the type of treatment received (F-statistic = 0.7and $p=0.6$) as shown in **Table 6.17**. At day 28, the overall mean decrease in total bilirubin was 3.5 μmol/L (95% CI; 2.5 to 4.5 $p<0.001$) over the baseline level and not associated with the type of treatment received (F-statistic = 0.9 and $p=0.4$). Similarly, the mean decreases in both the direct and indirect bilirubin concentrations over the baseline were significant on days 14 and 28 but were not associated with the type of treatment received. The mean changes in the direct and indirect bilirubin concentrations at days 14 and 28 after the start of treatment relative to baseline values according to treatment groups are shown in **Tables 6.18 and 6.19**. The mean changes in bilirubin remained unchanged when baseline parasite density, parity or gravidity were included in the regression model.

Table 6.17: - Mean changes in total bilirubin concentrations at days 14 and 28 after treatment according to treatment group.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p-value)
DAY 14							
CQ	-3.7	(18.4)	(-207 to 78.6)				0.7(0.6)
AQ	-3.4	(10.8)	(-64 to 45)	0.3	(-2.4 to 2.9)	0.84	
SP	-4.7	(11.6)	(-70.8 to 25.2)	-1.0	(-3.6 to 1.6)	0.45	
AQ+SP	-2.8	(10.3)	(-78.9 to 28.2)	0.9	(-1.8 to 3.5)	0.52	
DAY 28							0.8(0.5)
CQ	-4.6	(18.4)	(-218 to 29.4)				
AQ	-3.7	(12.2)	(-72 to 39)	0.9	(-1.9 to 3.7)	0.54	
SP	-3.6	(13.6)	(-79.8 to 37.6)	1.0	(-1.9 to 3.8)	0.51	
AQ+SP	-2.3	(10.9)	(-93 to 33.6)	2.3	(-0.6 to 5.1)	0.12	

Figure 6.14: - Mean total bilirubin levels at enrolment and at days 14 and 28 after start of treatment according to treatment group.

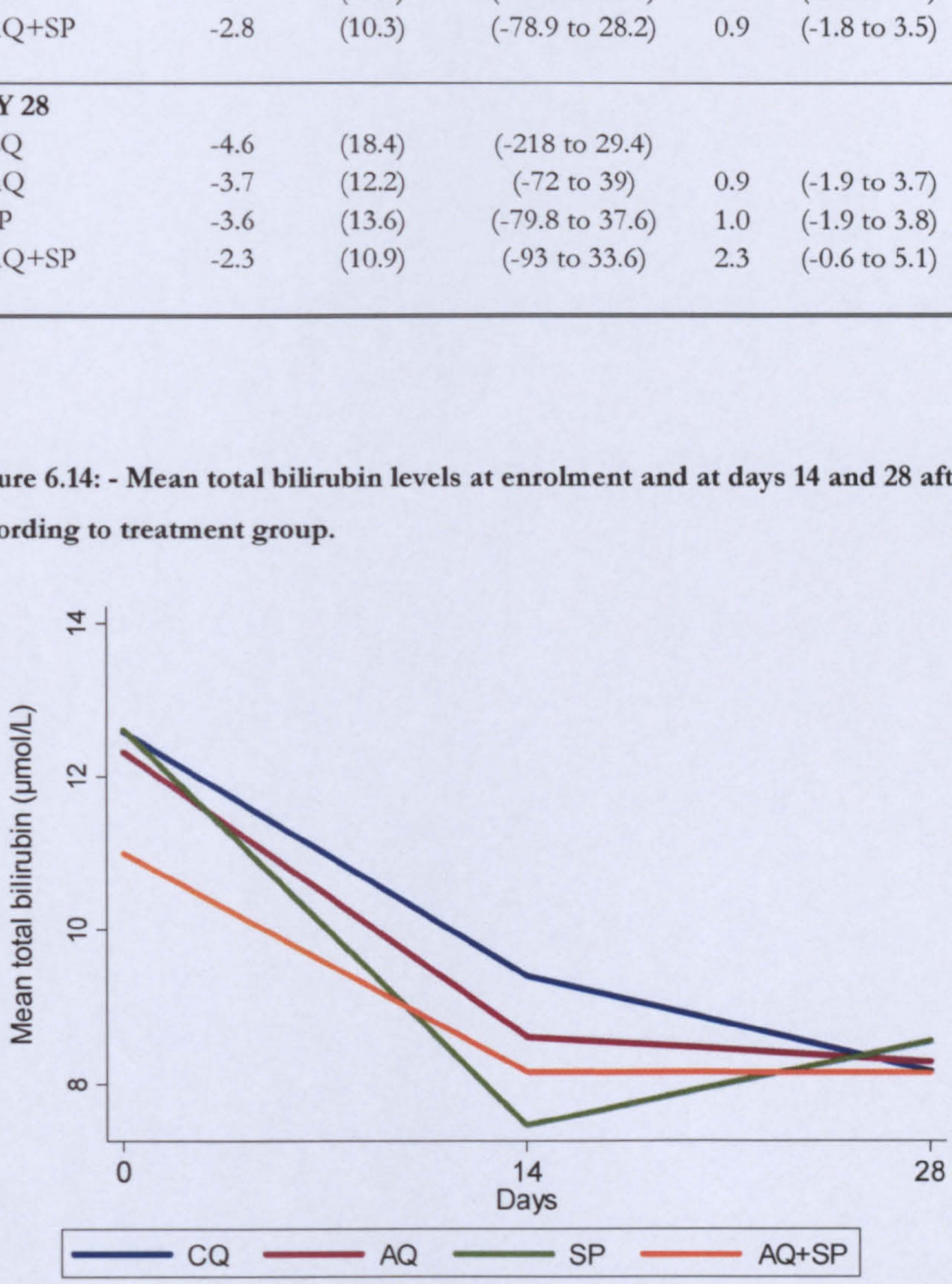


Table 6.18: - Mean changes in direct bilirubin concentrations at days 14 and 28 after treatment according to treatment group.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p-value)
DAY 14							
CQ	-1.7	(6.6)	(-61 to 41.3)				0.9(0.4)
AQ	-1.6	(5.3)	(-33 to 12.6)	0.1	(-1 to 1.2)	0.91	
SP	-1.6	(5.1)	(-35.8 to 16.9)	0.1	(-1 to 1.2)	0.88	
AQ+SP	-0.9	(5.1)	(-38.3 to 19.2)	0.8	(-0.3 to 1.2)	0.16	
DAY 28							
CQ	-1.6	(6.9)	(-68 to 24.2)				0.8(0.5)
AQ	-1.5	(6.1)	(-33 to 20.4)	0.1	(-1.2 to 1.4)	0.88	
SP	-1.4	(6.6)	(-39.8 to 33.9)	0.2	(-1.1 to 1.4)	0.81	
AQ+SP	-0.7	(5.4)	(-42.3 to 28.8)	0.9	(-0.4 to 2.2)	0.17	

Figure 6.15: - Mean direct bilirubin levels at enrolment and at days 14 and 28 after start of treatment according to treatment group.

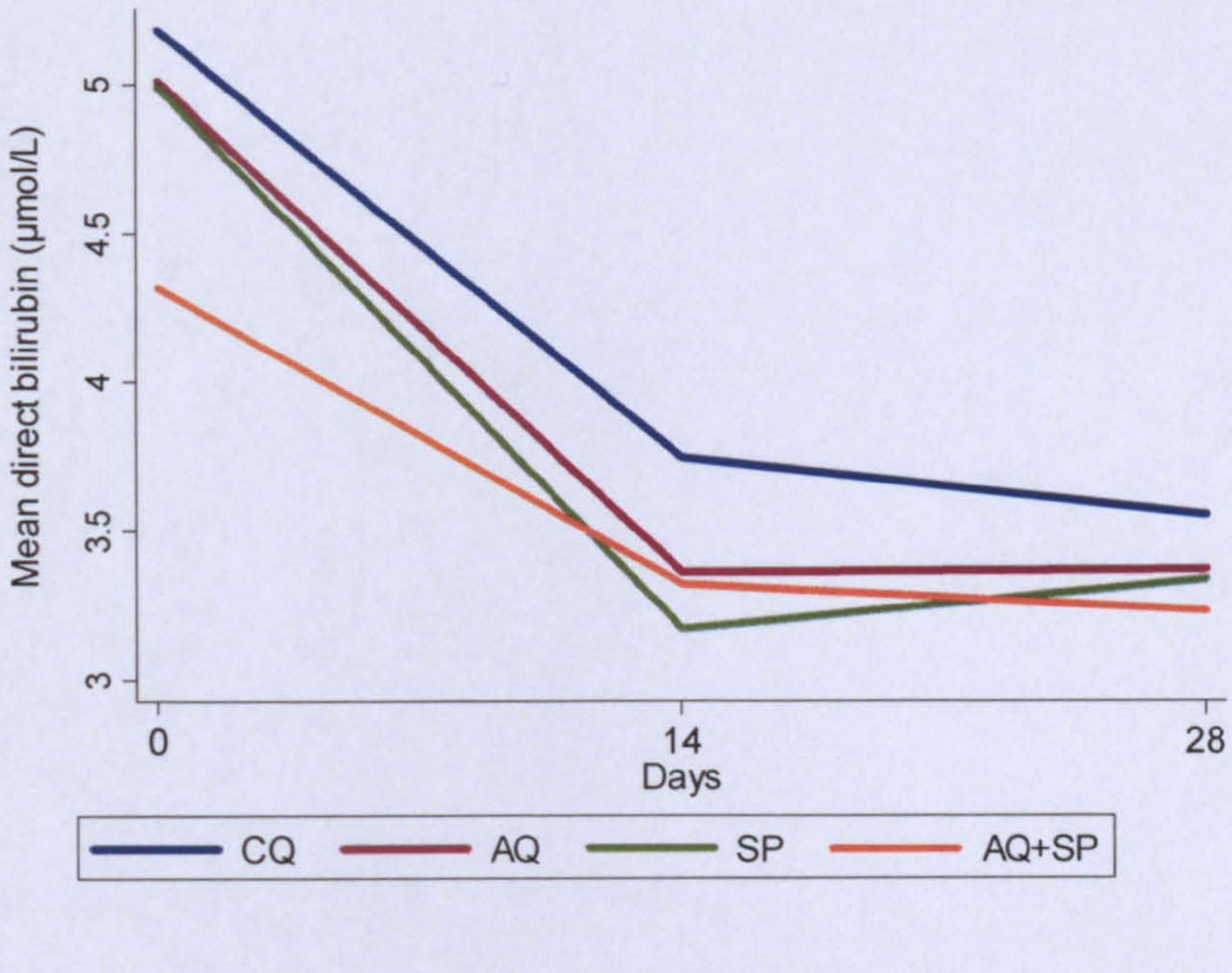
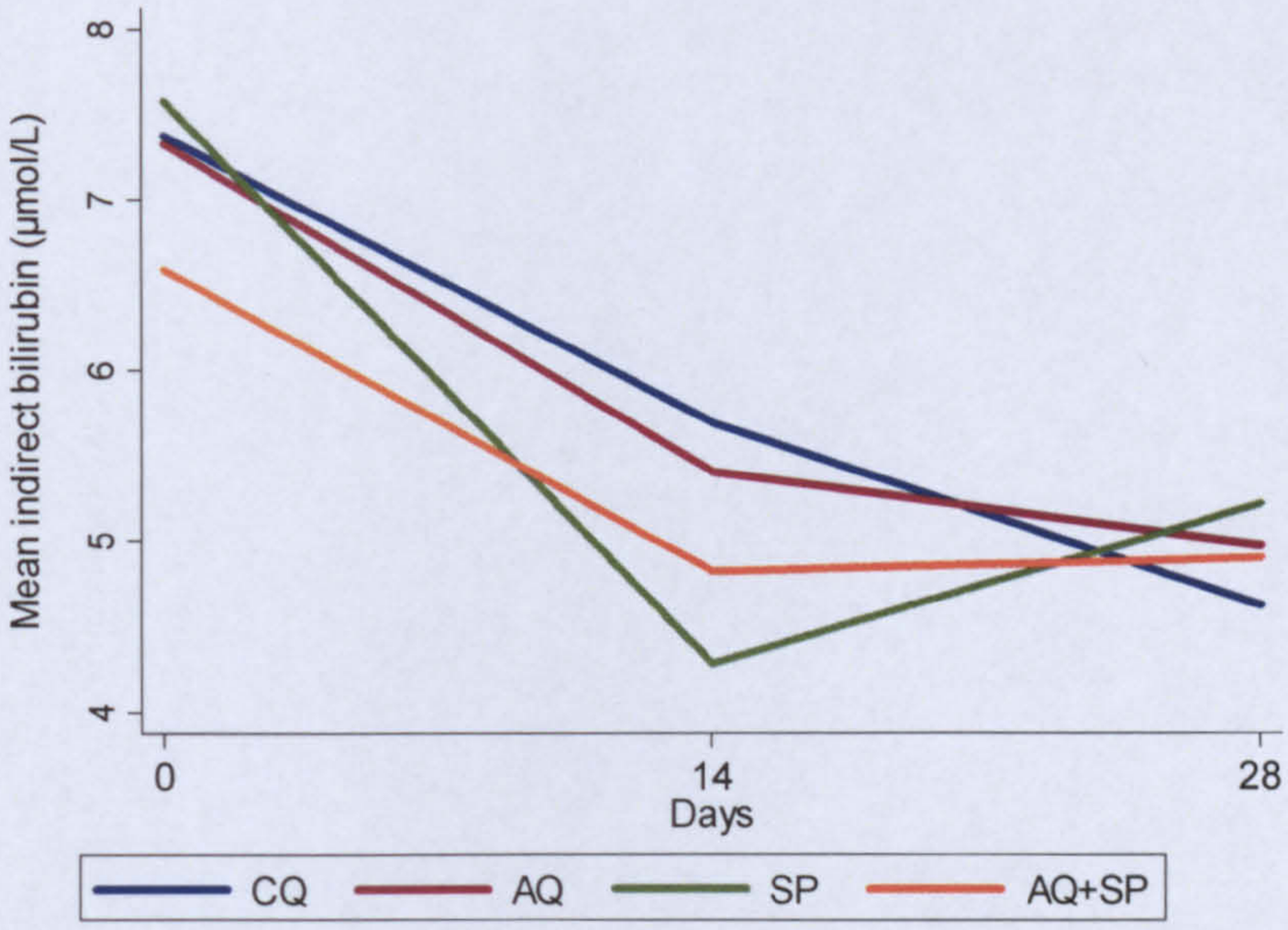


Table 6.19: - Mean changes in indirect bilirubin concentrations at days 14 and 28 after treatment according to treatment group.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p-value)
DAY 14							
CQ	-1.9	(12.9)	(-146 to 42.2)				0.9(0.5)
AQ	-1.7	(7.7)	(-35 to 37.1)	0.2	(-1.6 to 2)	0.81	
SP	-3.0	(7.7)	(-43 to 13.2)	-1.1	(-2.9 to 0.7)	0.24	
AQ+SP	-1.9	(7.2)	(-56 to 22.3)	0.1	(-1.7 to 1.9)	0.93	
DAY 28							
CQ	-2.9	(12.7)	(-150 to 28.4)				0.7(0.6)
AQ	-2.1	(7.8)	(-43 to 23.9)	0.8	(-1 to 2.7)	0.38	
SP	-2.1	(9.2)	(-58.5 to 31.9)	0.8	(-1.1 to 2.7)	0.41	
AQ+SP	-1.5	(7.1)	(-67.3 to 16.6)	1.4	(-0.5 to 3.3)	0.15	

Figure 6.16: - Mean indirect bilirubin levels at enrolment and at days 14 and 28 after start of treatment according to treatment group.



6.5 White blood cell (WBC) counts

The levels of total white cell counts at enrolment and at follow up time points after treatment are shown in **Table 6.20**. At baseline about 31% of the women had total white cell counts below 4 x 10^9/L but this proportion increased slightly at all the follow up days apart from at day 7. However, there were no associations between these changes and the treatment groups. The lowest WBC count in the study women after treatment was 1.6 x 10^9/L on day 3 in the CQ group. The lowest WBC count in women in the AQ group was 1.8 x 10^9/L on day 14 after treatment.

The overall changes in white cell counts after the start of treatment were investigated using paired t-test. Applying the nonparametric signed-rank test yielded the same conclusions and ANOVA was used to test for differences in the means of treatment groups. The overall mean changes in the total and differential white cell counts at days 3, 7, 14 and 28 after the start of treatment relative to baseline counts, are shown in **Tables 6.21**. The total white cell counts decreased relative to baseline levels on days 3, 14 and 28. The neutrophil counts increased while lymphocyte counts decreased after the start of treatment relative to baseline levels at all follow up days. Monocytes and eosinophils also showed a general decrease relative to baseline counts. However, as shown **Table 6.22,** the post treatment means are similar in all the treatment groups and the overall changes observed were not associated with the type of treatment received.

Table 6.20: - White blood cell (WBC) counts at enrolment and at follow up after start of treatment according to treatment group.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DAY 0										
< 4 x 10^9	85	(37.8)	68	(30.2)	60	(26.7)	70	(31.1)	283	(31.4)
4 – 11 x 10^9	140	(62.2)	156	(69.3)	165	(73.3)	155	(68.9)	616	(68.4)
>11 x 10^9	0	(0)	1	(0.5)	0	(0)	0	(0)	1	(0.2)
Mean (SD)	4.5	(1.4)	4.8	(1.5)	4.7	(1.4)	4.8	(1.4)	4.7	(1.4)
Range (min to max)	(2 - 10.6)		(2 - 11.7)		(1.5 - 10.9)		(2 - 9.6)		(1.5 - 11.7)	
DAY 3										
< 4 x 10^9	76	(35.5)	88	(40.4)	96	(44.2)	75	(34.4)	335	(38.6)
4 – 11 x 10^9	138	(64.5)	130	(59.6)	119	(54.8)	142	(65.1)	529	(61)
>11 x 10^9	0	(0)	0	(0)	2	(1)	1	(0.5)	3	(0.4)
Mean (SD)	4.6	(1.5)	4.6	(1.5)	4.4	(1.6)	4.7	(1.5)	4.6	(1.5)
Range (min to max)	(1.6 - 10.5)		(2 - 10.2)		(2 - 13.1)		(2 - 11.4)		(1.6 - 13.1)	
DAY 7										
< 4 x 10^9	62	(28.8)	71	(33.3)	69	(32.2)	51	(23.9)	253	(29.6)
4 – 11 x 10^9	151	(70.2)	142	(66.7)	144	(67.3)	162	(76.1)	599	(70)
>11 x 10^9	2	(1)	0	(0)	1	(0.5)	0	(0)	3	(0.4)
Mean (SD)	4.9	(1.6)	4.8	(1.6)	4.7	(1.5)	4.8	(1.4)	4.8	(1.5)
Range (min to max)	(2 - 12.1)		(2 - 10.9)		(2 - 15.2)		(2 - 9.9)		(2 - 15.2)	
DAY 14										
< 4 x 10^9	77	(36.2)	71	(32.7)	78	(36.8)	76	(35.5)	302	(35.3)
4 – 11 x 10^9	135	(63.4)	146	(67.3)	134	(63.2)	138	(64.5)	553	(64.6)
>11 x 10^9	1	(0.5)	0	(0)	0	(0)	0	(0)	1	(0.1)
Mean (SD)	4.7	(1.7)	4.5	(1.3)	4.6	(1.6)	4.4	(1.4)	4.6	(1.5)
Range (min to max)	(1.9 - 18)		(1.8 - 9.3)		(2 - 10.8)		(2.1 - 9.2)		(1.8 - 18)	
DAY 28										
< 4 x 10^9	73	(35.6)	73	(34.1)	76	(37.1)	77	(37)	299	(36)
4 – 11 x 10^9	132	(64.4)	141	(65.9)	129	(62.9)	130	(62.5)	532	(63.9)
>11 x 10^9	0	(0)	0	(0)	0	(0)	1	(0.5)	1	(0.1)
Mean (SD)	4.4	(1.3)	4.5	(1.4)	4.5	(1.5)	4.5	(1.4)	4.5	(1.4)
Range (min to max)	(2 - 8.8)		(2.2 - 10.5)		(2 - 10.7)		(2 - 13.3)		(2 - 13.3)	

Table 6.21: - Mean changes in differential white cell counts at follow up after the start of treatment.

	N	Overall mean difference (SD)		95% CI	p-value
Total WBC					
DAY 3	867	-0.1	(1.7)	(-0.2 to -0.01)	0.03
DAY 7	855	0.1	(1.8)	(-0.01 to 0.2)	0.08
DAY 14	856	-0.1	(1.8)	(-0.3 to -0.03)	0.02
DAY 28	832	-0.2	(1.8)	(-0.3 to -0.1)	<0.001
Neutrophil					
DAY 3	867	0.6	(8.7)	(-0.01 to 1.1)	0.05
DAY 7	856	1.3	(9.5)	(0.7 to 1.9)	<0.001
DAY 14	856	1.4	(9.5)	(0.8 to 2.0)	<0.001
DAY 28	832	1.7	(8.8)	(1.1 to 2.3)	<0.001
Lymphocytes					
DAY 3	867	-0.5	(8.9)	(-1.1 to 0.1)	0.1
DAY 7	856	-1.1	(9.6)	(-1.8 to -0.5)	<0.001
DAY 14	856	-1.1	(9.1)	(-1.7 to -0.5)	<0.001
DAY 28	832	-1.3	(8.9)	(-1.9 to -0.7)	<0.001
Monocytes					
DAY 3	867	-0.02	(0.6)	(-0.1 to 0.01)	0.3
DAY 7	856	-0.01	(0.6)	(-0.04 to 0.03)	0.7
DAY 14	856	0.03	(1.5)	(-0.06 to 0.1)	0.5
DAY 28	832	-0.01	(0.6)	(-0.1 to 0.03)	0.5
Eosinophils+Basophils					
DAY 3	867	0.02	(2.6)	(-0.2 to 0.2)	0.8
DAY 7	856	-0.1	(2.7)	(-0.3 to 0.1)	0.4
DAY 14	856	-0.2	(2.7)	(-0.4 to -0.02)	0.03
DAY 28	832	-0.3	(2.4)	(-0.5 to -0.2)	<0.001

Table 6.22: - Mean differential white cell counts expressed as the percentage of total WBC (%WBC)

		Neutrophils		Lymphocytes		Eosinophils plus Basophils		Monocytes	
	N	% WBC	(SD)	% WBC	(SD)	% WBC	(SD)	% WBC	(SD)
DAY 0									
CQ	225	62.3	(6.6)	35.6	(6.5)	2.1	(2.1)	0.1	(0.4)
AQ	225	62.7	(7.5)	34.5	(7.6)	2.1	(1.9)	0.2	(0.5)
SP	225	61.9	(6.6)	36.1	(6.5)	1.9	(1.8)	0.1	(0.4)
AQ+SP	225	63.4	(6.5)	34.6	(6.5)	2.0	(2.0)	0.1	(0.3)
Total	900	62.6	(6.8)	35.2	(6.8)	2.0	(1.9)	0.1	(0.4)
p-value		**0.1**		**0.04**		**0.3**		**0.05**	
DAY 3									
CQ	214	63.4	(7.3)	34.4	(7.1)	2.1	(2.1)	0.1	(0.3)
AQ	218	62.7	(7.1)	35.3	(6.9)	1.9	(1.8)	0.1	(0.5)
SP	217	62.4	(7.6)	35.3	(7.2)	2.2	(2.4)	0.1	(0.4)
AQ+SP	218	63.8	(6.3)	34.1	(6.5)	2.0	(1.8)	0.1	(0.3)
Total	867	63.1	(7.1)	34.8	(7)	2.0	(2.0)	0.1	(0.4)
p-value		**0.2**		**0.2**		**0.3**		**0.2**	
DAY 7									
CQ	215	63.7	(8.2)	34.2	(8.1)	1.9	(1.8)	0.1	(0.4)
AQ	214	63.9	(8)	34.1	(7.9)	2.0	(2.3)	0.1	(0.3)
SP	214	63.6	(7.5)	34.2	(6.8)	2.0	(2.2)	0.1	(0.4)
AQ+SP	213	63.9	(7.2)	34.1	(7.2)	1.9	(2.1)	0.1	(0.5)
Total	856	63.8	(7.7)	34.1	(7.5)	1.9	(2.1)	0.1	(0.4)
p-value		**0.9**		**0.9**		**0.3**		**0.2**	
DAY 14									
CQ	213	64.4	(7.6)	33.5	(7.3)	2.0	(2.1)	0.1	(0.4)
AQ	217	63.9	(6.7)	34.2	(6.8)	1.8	(1.8)	0.3	(2.7)
SP	212	64.0	(7.4)	34.3	(7.4)	1.7	(1.7)	0.1	(0.4)
AQ+SP	214	63.4	(8.5)	34.5	(6.9)	1.7	(2.3)	0.1	(0.4)
Total	856	63.9	(7.6)	34.1	(7.1)	1.8	(2.0)	0.1	(1.4)
p-value		**0.6**		**0.5**		**0.9**		**0.5**	
DAY 28									
CQ	205	63.7	(7.4)	34.5	(7.2)	1.7	(1.9)	0.1	(0.5)
AQ	214	64.4	(7.2)	33.9	(6.9)	1.6	(1.6)	0.1	(0.3)
SP	205	63.9	(6.6)	34.3	(6.7)	1.7	(1.8)	0.1	(0.3)
AQ+SP	208	65.0	(6.7)	33.1	(6.5)	1.8	(1.9)	0.1	(0.4)
Total	832	64.3	(7)	33.9	(6.8)	1.7	(1.8)	0.1	(0.4)
p-value		**0.2**		**0.2**		**0.7**		**0.7**	

CHAPTER SEVEN

PREGNANCY OUTCOME

CHAPTER 7 PREGNANCY OUTCOME

7.1 Introduction

This chapter presents results on the parasitological and haematological outcomes at delivery and post partum in the study women. These outcomes are presented initially as the estimates of treatment effect and then as overall effect compared to baseline levels.

The chapter also includes a descriptive analysis of birth outcomes by treatment group. The overall incidences of adverse pregnancy outcomes in the study group are compared to local rates for abortion, stillbirth, congenital abnormality, and mean gestation at delivery obtained from St Theresa's Hospital's records.

7.2 Follow up at delivery and postpartum

A total of 827 women who completed treatment were expected to have supervised deliveries at a clinic, health centre or at the hospital. Records of deliveries conducted at various sites were obtained for 711 of those women (86%). There were 66 home deliveries assisted by relatives, 107 home deliveries assisted by traditional birth attendants, 114 deliveries at health centres and clinics and 422 deliveries at hospital. The distribution of these is similar between the treatment groups. The flow of participants after the initial day 28 follow up, through delivery to postpartum is shown in **Figure 7.1**. Delivery records are not available for 116 women who completed treatment; 41, 48, 51 and 49 in the CQ, AQ, SP and the AQ+SP combination groups respectively. Losses to follow up at delivery or postpartum were mainly due to migration outside the study area. Most of them claimed they needed the support of their families who lived outside the study area to care for them at delivery and/or at postpartum, and so travelled to be with their families before delivery.

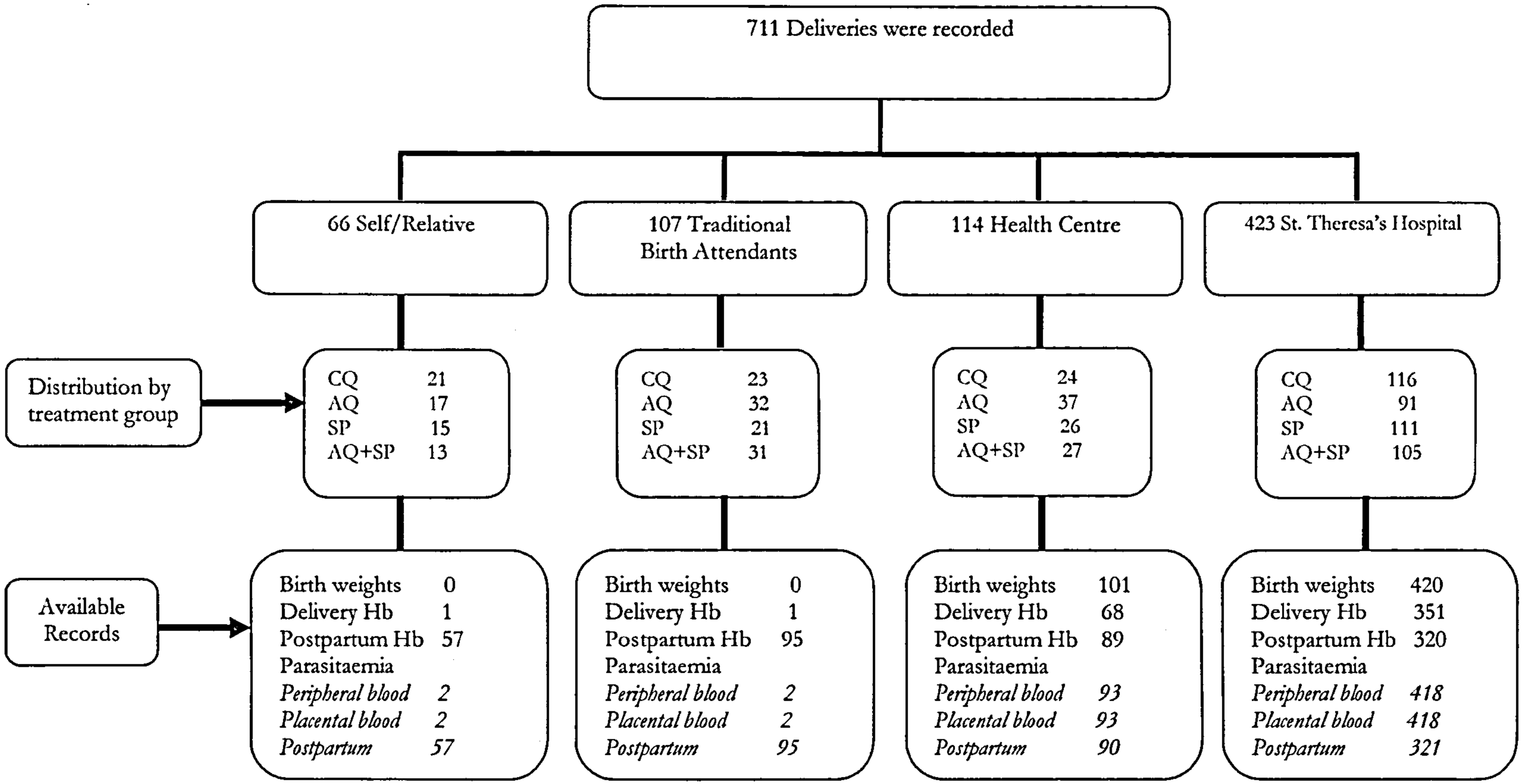


Figure 7.1: - Participants flow after the initial day 28 follow up to postpartum.

7.3 Parasitological outcomes at delivery and postpartum

The prevalence of asexual *P. falciparum* in peripheral blood, placental blood and placental impression smears at delivery and in peripheral blood post partum according to treatment group are shown in **Table 7.1**.

Table 7.1: - Parasite prevalence at delivery and post partum according to treatment group.

	Parasite prevalence			Unadjusted odds ratio (95% CI)		p-value	Adjusted odds ratio†† (95% CI)		p-value
	N	n	(%)						
AT DELIVERY									
Peripheral blood									
CQ	133	13	(9.8)	1.0			1.0		
AQ	125	10	(8)	0.8	(0.3 - 1.9)	0.6	0.9	(0.4 - 2.3)	0.9
SP	133	6	(4.5)	0.4	(0.2 - 1.2)	0.1	0.4	(0.1 - 1.1)	0.1
AQ+SP	124	3	(2.4)	0.2	(0.1 - 0.8)	0.02	0.3	(0.1 - 1.0)	0.06
Total	**515**	**32**	**(6.2)**						
Placental blood									
CQ	133	24	(18.1)	1.0			1.0		
AQ	125	12	(9.6)	0.5	(0.2 - 1.0)	0.05	0.5	(0.2 - 1.0)	0.06
SP	133	10	(7.5)	0.4	(0.2 - 0.8)	0.01	0.4	(0.2 - 0.8)	0.01
AQ+SP	124	13	(10.5)	0.5	(0.3 - 1.1)	0.1	0.5	(0.3 - 1.1)	0.1
Total	**515**	**59**	**(11.5)**						
Placental impression smear									
CQ	133	26	(19.6)	1.0			1.0		
AQ	125	16	(12.8)	0.6	(0.3 - 1.2)	0.2	0.6	(0.3 - 1.2)	0.2
SP	133	16	(12)	0.6	(0.3 - 1.1)	0.1	0.5	(0.3 - 1.0)	0.1
AQ+SP	124	18	(14.5)	0.7	(0.4 - 1.3)	0.3	0.7	(0.4 - 1.4)	0.4
Total	**515**	**76**	**(14.8)**						
AT POSTPARTUM									
CQ	152	5	(3.3)	1.0			1.0		
AQ	145	4	(2.8)	0.8	(0.2 - 3.2)	0.8	0.9	(0.2 - 3.5)	0.9
SP	138	10	(7.3)	2.3	(0.8 - 6.9)	0.1	2.5	(0.8 - 7.7)	0.1
AQ+SP	144	6	(4.2)	1.3	(0.4 - 4.3)	0.7	1.4	(0.4 - 4.7)	0.6
Total	**579**	**25**	**(4.3)**						

†† Odd ratios adjusted for parity, age, gestation at enrolment and duration between enrolment and delivery.

7.3.1 Parasite prevalence at delivery

These analyses compared parasite prevalence at delivery in peripheral blood, placental blood and placental impression smear in the AQ, SP and AQ+SP combination groups with that in the CQ group in each sample.

Peripheral blood parasitaemia

As shown in **Table 7.1**, the prevalences of asexual *P. falciparum* parasitaemia were 9.8%, 8%, 4.5% and 2.4% for the CQ, AQ, SP and the AQ+SP combination groups respectively in the peripheral blood. The ORs of parasitaemia was 0.8 (95% CI; 0.3 to 1.9 p=0.6) in the AQ group, 0.4 (0.2 to 1.2 p=0.1) in the SP group and 0.2 (95% CI; 0.1 to 0.8 p=0.02) in the AQ+SP combination group compared to the CQ group. The ORs did not change significantly after adjustments were made for baseline parasite density, gestation at enrolment, duration between enrolment and delivery, parity and age. Thus all the test drugs have greater protective efficacy against parasitaemia at delivery compared to CQ, but it is only in the AQ+SP combination group that the effect was statistically significant. Peripheral blood parasitaemia was significantly associated with baseline parasite density. The unadjusted ORs were 3.2 (95% CI; 1.5 to 7.1 p=0.004) and 2.9 (95% CI 1.3 to 6.5 p=0.01) after adjustment were for gestation at enrolment, duration between enrolment and delivery, parity and age.

Placental blood parasitaemia

As shown in **Table 7.1**, the prevalences of asexual *P. falciparum* parasitaemia were 18.1%, 9.6%, 7.5% and 10.5% for the CQ, AQ, SP and the AQ+SP combination groups respectively in the placental blood. The ORs of parasitaemia was 0.5 (95% CI; 0.2 to 1.0 p=0.05) in the AQ group, 0.4 (0.2 to 0.8 p=0.01) in the SP group and 0.5 (95% CI; 0.3 to 1.1 p=0.09) in the AQ+SP combination group compared to the CQ group. The ORs did not change significantly after adjustments for baseline parasite density, gestation at enrolment, duration between enrolment and delivery, parity and age. Thus the protective efficacy against placental blood parasitaemia at delivery was significantly greater in the AQ and SP groups compared to CQ, but only of borderline significance in the AQ+SP combination group. Placental blood parasitaemia was not associated with baseline parasite density, gestation at enrolment, duration between enrolment and delivery, parity or age.

Placental impression smear

As shown in **Table 7.1**, the prevalences of asexual *P. falciparum* parasitaemia were 19.6%, 12.8%, 12% and 14.5% for the CQ, AQ, SP and the AQ+SP combination groups respectively in the placental impression smear. The ORs of parasite prevalence was 0.6 (95% CI; 0.3 to 1.2 p=0.2) in the AQ group, 0.6 (95% CI; 0.3 to 1.1 p=0.1) in the SP group and 0.7 (95% CI; 0.4 to 1.3 p=0.3) in the AQ+SP combination group compared to the CQ group. The ORs did not change after adjustments for baseline parasite density, gestation at enrolment, duration between enrolment and delivery, parity and age. Thus using the placental impression smear, the protective effects of the test drugs against placental parasitaemia were not significantly different from that of CQ. Placental parasitaemia by impression smear was not associated with baseline parasite

density unadjusted and when adjusted for gestation at enrolment, duration between enrolment and delivery, parity and age.

7.3.2 Postpartum parasite prevalence

As shown in **Table 7.1**, the prevalences of asexual *P. falciparum* parasitaemia were 3.3%, 2.8%, 7.3% and 4.2% for the CQ, AQ, SP and the AQ+SP combination groups respectively at postpartum. The ORs of parasitaemia was 0.8 (95% CI; 0.2 to 3.2 p=0.8) in the AQ group, 2.3 (95% CI; 0.8 to 6.9 p=0.1) in the SP group and 1.3 (95% CI; 0.4 to 4.3 p=0.7) in the AQ+SP combination group compared to the CQ group. The ORs did not change significantly after adjustments for baseline parasite density, gestation at enrolment, duration between enrolment and delivery, parity and age. Thus the protective effects of the test drugs against postpartum parasitaemia were not significantly different from that of CQ. Postpartum parasitaemia was not associated with baseline parasite density unadjusted and when adjusted for gestation at enrolment, duration between enrolment and delivery, parity and age.

7.4 Haematological outcomes at delivery and postpartum

The proportions of study pregnant women in 3 categories of haemoglobin concentrations at delivery and postpartum are shown in **Table 7.2**. There is a significant improvement in Hb levels within all treatment groups at delivery and postpartum treatment as illustrated by **Figure 7.2**. The proportions of women with Hb equal to 11g/dl or above had increased from about 10% at enrolment to 42% and 77% at delivery and postpartum respectively while the proportions of those with Hb below 8g/dl decreased over the period. There was a 1.1g/dl (0.9 – 1.3) $p<0.001$ increase in Hb over baseline level at delivery but has no apparent association with the type of treatment (F-statistic = 1.5; $p=0.2$). At postpartum, the increase over baseline mean Hb was 2.4g/dl (2.3 – 2.6) $p<0.001$ with a borderline association with the mean changes between the groups (F-statistic = 2.5; $p=0.06$). The change in mean Hb at postpartum tends to be higher than would have been at delivery within the treatment groups as shown in **Figure 7.3**.

Table 7.2: - Haemoglobin concentrations at delivery and at six weeks postpartum.

	CQ		AQ		SP		AQ+SP		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
DELIVERY Hb										
< 8g/dl	17	(15.0)	15	(14.9)	14	(13.0)	15	(15.1)	61	(14.5)
8 - 10.9g/dl	57	(50.4)	40	(39.6)	50	(46.3)	37	(37.4)	184	(43.7)
11g/dl or above	39	(34.5)	46	(45.5)	44	(40.7)	47	(47.5)	176	(41.8)
Mean (SD)	10.1	(2.2)	10.4	(2.1)	10.2	(1.9)	10.4	(2.1)	10.3	(2.1)
Median (IQR)	10.3	(3.0)	10.7	(2.9)	10.5	(2.6)	10.7	(2.9)	10.5	(2.9)
POSTPARTUM Hb										
< 8g/dl	1	(0.6)	1	(0.7)	0	(0)	0	(0)	2	(0.3)
8 - 10.9g/dl	43	(28.5)	34	(23.6)	31	(22.5)	24	(16.7)	132	(22.9)
11g/dl or above	107	(70.9)	109	(75.7)	107	(77.5)	120	(83.3)	443	(76.8)
Mean (SD)	11.6	(1.6)	11.5	(1.5)	11.8	(1.6)	11.8	(1.3)	11.7	(1.5)
Median (IQR)	12.0	(3)	12.0	(1)	12.0	(2)	12.0	(1.5)	12.0	(2)

Figure 7.2: - Comparison of delivery and postpartum haemoglobin concentrations to baseline levels according to treatment group.

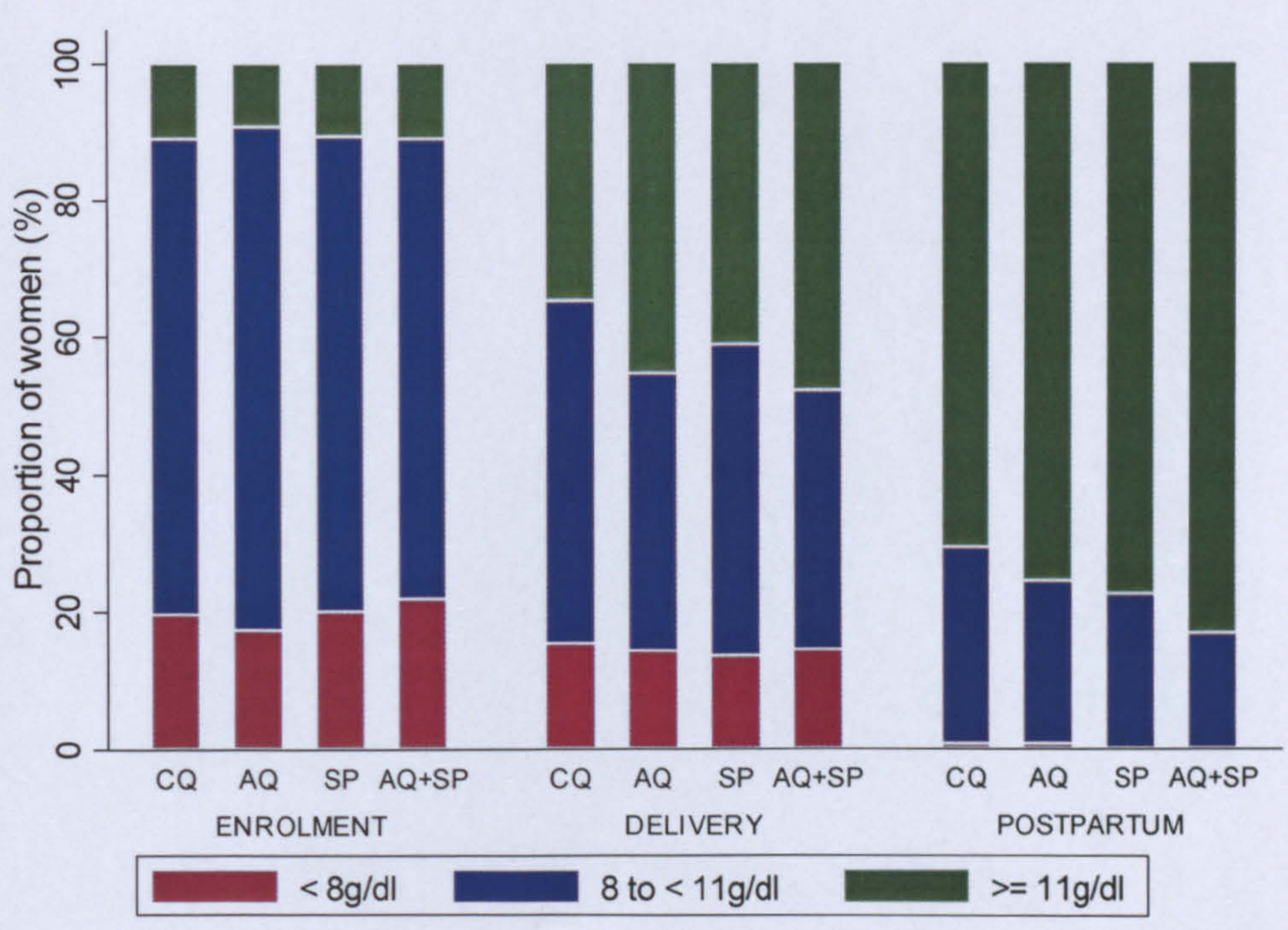
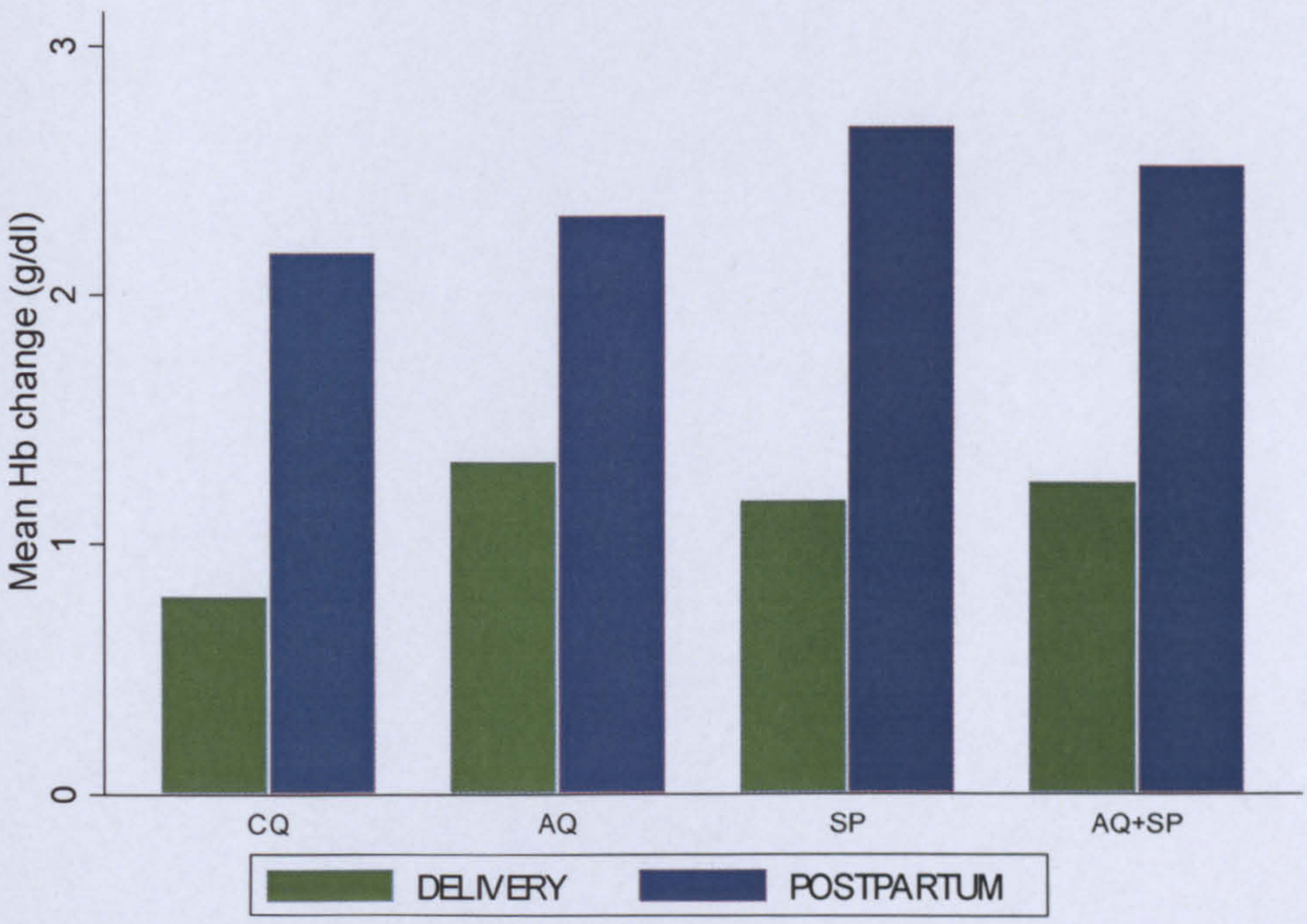


Table 7.3: - Mean changes in Hb concentrations at delivery and postpartum relative to baseline concentrations.

	Mean change (SD)		Range (min to max)	Mean difference compared to CQ (95% CI)		p - value	F-statistic (p-value)
DELIVERY							
CQ	0.8	(2.2)	(-5.4 - 6.4)				1.5(0.2)
AQ	1.3	(2.0)	(-3.8 - 7)	0.6	(-0.01 - 1.1)	0.06	
SP	1.2	(2.0)	(-3.8 - 5.9)	0.4	(-0.1 -1.0)	0.1	
AQ+SP	1.2	(2.3)	(-5.4 - 7.4)	0.5	(-0.1 - 1.0)	0.1	
POSTPARTUM							
CQ	2.2	(1.8)	(-2.4 - 7.9)				2.3(0.08)
AQ	2.3	(1.7)	(-2.3 - 8)	0.15	(-0.3 - 0.6)	0.47	
SP	2.7	(1.9)	(-2.3 - 7.6)	0.51	(0.1 - 0.9)	0.02	
AQ+SP	2.5	(1.7)	(-1.1 - 8)	0.35	(-0.06 - 0.8)	0.09	

Figure 7.3: - Mean Hb increase over baseline according to treatment group at delivery and postpartum.



7.5 Birth weights

Birth weight records were available for 101 and 420 deliveries conducted at health centres and the hospital respectively. Overall the incidence of low birth weight was 12.7% as shown in **Table 7.4**. The mean birth weigh was 2898g ranging from 1000 to 4300g for women who were treated only once. It was 2846g ranging from 1000 to 4000g in those who had retreatment. In separate analyses of data in women treated for single and double episodes of malaria, no differences were found. The results of the overall incidence of low birth weights according to treatment groups are shown in **Table 7.4**.

Table 7.4: - Birth weights of babies born to study pregnant women according to treatment group.

	BIRTH WEIGHT								
	>/=2500 g		<2500 g		Mean (SD)	Median (IQR)		Range	
	n	*(%)*	*n*	*(%)*				(min to max)	
TREATMENT									
CQ	117	(86.7)	18	(13.3)	2919	(495)	3000	(500)	(1000 - 4200)
AQ	104	(83.2)	21	(16.8)	2866	(579)	3000	(400)	(1000 - 4000)
SP	118	(88.1)	16	(11.9)	2874	(479)	3000	(600)	(1300 - 4000)
AQ+SP	116	(91.3)	11	(8.7)	2931	(480)	3000	(600)	(1000 - 4300)
Total	455	(87.3)	66	(12.7)	2898	(508)	3000	(500)	(1000 - 4300)

7.6 Pregnancy outcomes

No maternal deaths were recorded. The numbers of known neonatal outcomes according to treatment groups are shown in **Table 7.5.**

Table 7.5: - Neonatal outcomes according to treatment groups.

	CQ		AQ		SP		AQ+SP		Total	
Neonatal Outcomes	*n*	*(%)*	*n*	*(%)*	*n*	*(%)*	*n*	*(%)*	*n*	*(%)*
Abortions	1	(0.5)	0	(0)	2	(1.2)	0	(0)	3	(0.4)
Still births	2	(1.1)	1	(0.6)	2	(1.2)	1	(0.6)	6	(0.8)
Perinatal deaths	2	(1.1)	3	(1.7)	4	(2.3)	1	(0.6)	10	(1.4)
Neonatal deaths	3	(1.6)	2	(1.1)	1	(0.6)	2	(1.1)	8	(1.1)
Alive	176	(95.7)	171	(96.6)	165	(94.8)	172	(97.7)	684	(96.2)

7.6.1 Abortions

As shown in **Table 7.5,** two abortions were recorded by midwives. The first event was in a 19 year old primigravid enrolled on 8/7/03 at 18 weeks gestation who received SP. A TBA reported she aborted on 21/7/03. Her parasite density at enrolment was 720/μL. The second event was in 18 year old primigravid enrolled on 27.01/04 at 19 weeks gestation who received CQ. Her baseline parasite density was 15200/μL. She aborted at the St. Theresa's Hospital on 3/2/04. Another woman's outcome was recorded as still birth but by the study definitions should have been recorded as an abortion. She was a 41 year old with parity of 6 and enrolled on 10/02/04 at 20 weeks gestation who received SP. Her baseline parasite density was 1240/μL. She aborted at the St. Theresa's Hospital on 23/2/04.

7.6.2 Still births

As shown in **Table 7.5**, there were six still births; 2 in the CQ group, 1 each in the AQ and AQ+SP groups and 2 in the SP group.

7.6.3 Preterm deliveries

There are records of 97 preterm deliveries; 27 (14.7%), 30 (17%), 18 (10.3%) and 22 (12.5%) in the CQ, AQ, SP and AQ+SP groups respectively. The groups were not statistically different. Two of the preterm babies died at birth.

7.6.4 Perinatal deaths

There were records of ten perinatal deaths; 2, 3, 4 and 1 in the CQ, AQ, SP and AQ+SP groups respectively as shown in **Table 7.5**. Two of these were home deliveries, 3 occurred at health centres and 6 at St. Theresa's hospital. Midwives reported they all needed resuscitation at birth. They all had birth weights 3kg or above apart from one preterm baby.

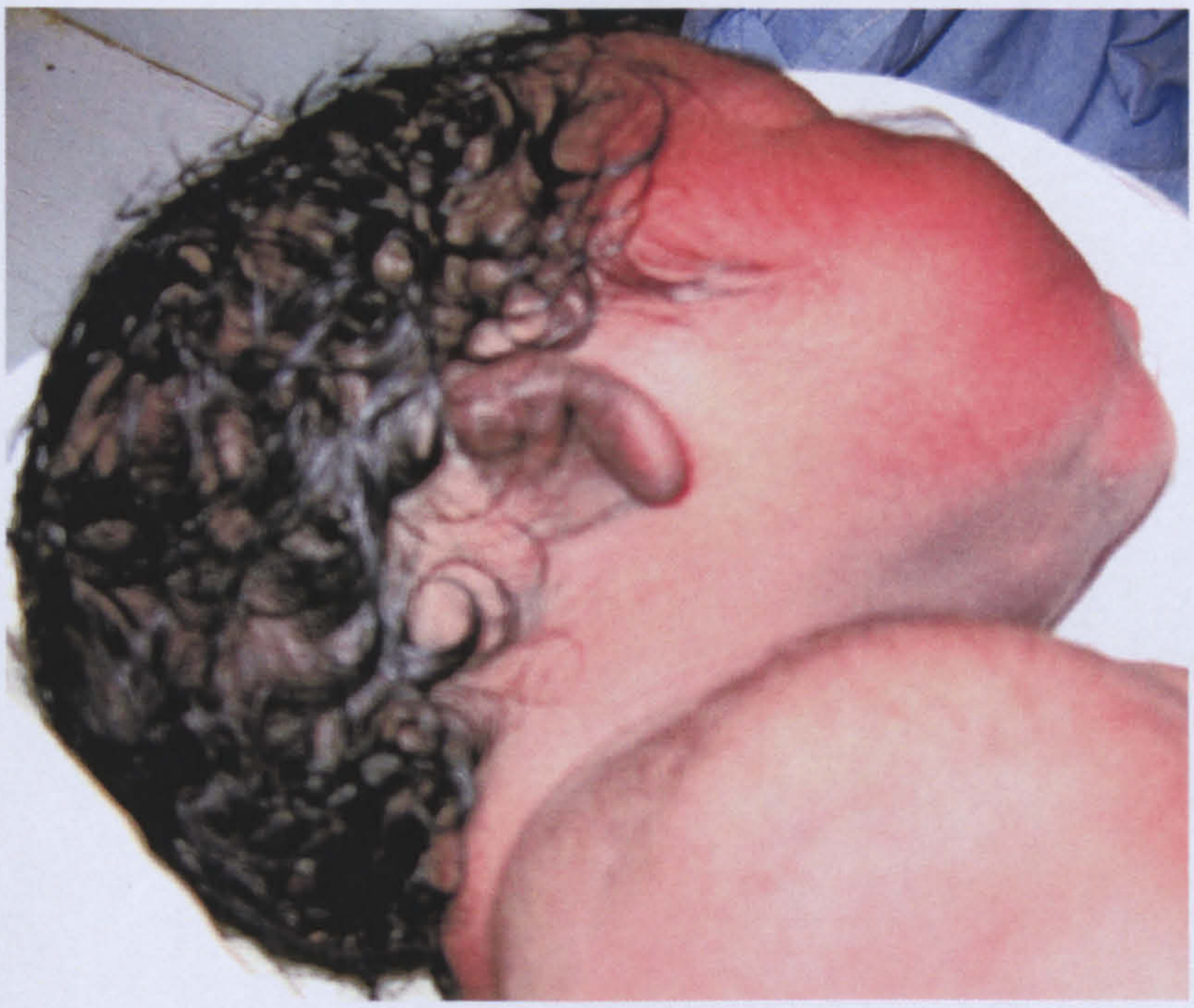
7.6.5 Neonatal deaths

There were eight neonatal deaths recorded between one and four weeks of the postpartum period. These include 3 (1.6%), 2 (1.1%), 1 (0.6%), and 2 (1.1%) in the CQ, AQ, SP and AQ+SP groups respectively as shown in **Table 7.5**. The groups were not statistically different.

7.6.6 Abnormalities

There are records of 8 abnormalities. Seven of the babies had extra digits and one shown in **Figure 7.4** below had a malformed ear.

Figure 7.4: - A new born with malformed left ear.



This baby was born to a 25 year old primigravid and a teacher. She received CQ at 22 weeks of gestation. She was delivered by caesarean section due to pre-eclampsia. No other abnormality was detected on the baby. The ear has since been reconstructed.

CHAPTER EIGHT

DISCUSSION AND CONCLUSION

CHAPTER 8 DISCUSSION AND CONCLUSION

8.1 Introduction

The present study was conducted in a routine antenatal clinic setting to compare the efficacy, safety and tolerability of AQ and SP as single or combination therapies with CQ in the treatment of *P. falciparum* infection in pregnancy. The key findings on epidemiology of malaria during pregnancy, performance of screening for malaria parasitaemia at routine antenatal clinics, and the efficacy, safety and tolerance of the study drugs are discussed in this chapter. Also discussed, are the implications of the findings for research, policy and practice.

8.2 Local epidemiology of malaria in pregnancy

The prevalence of antenatal malaria detected by OptiMAL® antigen testing in the study area was about 21% with no significant seasonal variation. The main parasite species found was *P. falciparum*. Antenatal malaria was significantly associated with young age, nulliparity or primiparity and anaemia (Hb below 11g/dl). These features are consistent with the epidemiologic patterns of malaria in pregnancy found in other malaria endemic regions with perennial malaria transmission (Nair and Nair 1993; Steketee *et al.* 2001; Zhou *et al.* 2002; Bouyou Akotet *et al.* 2003). This pattern was very similar among the study women when the association between their baseline parasite densities and parity, age and gravidity were examined. This is expected since the study sample came from the same population. Among the study women, the younger, nulliparous or primiparous women had significantly higher parasite densities and lower haemoglobin levels than older or multiparous women. About 90% of the study women had anaemia (Hb < 11g/dl) and a fifth of them had severe anaemia (Hb < 7g/dl). Anaemia was associated significantly with parasite density, age and parity. These findings agree with those from earlier studies that have investigated the causes of anaemia in pregnancy (Matteelli *et al.* 1994; Shulman *et al.* 1996;

Ndyomugyenyi and Magnussen 1999; Mockenhaupt *et al.* 2000). Anaemia in the present study was also associated with high levels of bilirubin (direct and indirect) suggesting haemolysis as the mechanism. Previous studies have linked hypohaptoglobinaemia in pregnant women with malaria indicating haemolysis as a cause of maternal anaemia in pregnancy (Gilles *et al.* 1969; Rougemont *et al.* 1988; Yerly *et al.* 1990). Many other studies have attributed maternal anaemia in pregnancy to other factors such as iron, folate, vitamin A, vitamin B12 deficiencies, HIV/AIDS, haemoglobinopathies and hookworm infestations (Liljestrand *et al.* 1986; Fleming 1989; van den Broek 1996; van den Broek 1998; Verhoeff *et al.* 1999). In the present study, anaemia was not associated with prior chloroquine use, hookworm infestation or hepatosplenomegaly. The other causes of anaemia in pregnancy including iron and folate deficiencies, HIV/AIDS and haemoglobinopathies were not investigated. However, Mockenhaupt and others showed that apart from homozygous alpha thalassaemia, iron deficiency and haemoglobinopathies did not have significant associations with anaemia in Ghanaian pregnant women (Mockenhaupt *et al.* 2000).

8.3 Performance of the OptiMAL® antigen test

These are the first data describing the performance characteristics of the dipstick for routine use during antenatal clinic sessions. A potential limiting factor in assessing the performance of the antigen test was the choice of slide microscopy as the "gold standard" as its performance depends heavily on the expertise of the microscopist. This study increased the chances of reliable microscopy by employing the services of an experienced microscopist. In addition, an independent assessor blindly assessed random samples throughout the study period. Both microscopists were blinded to the dipstick results. Only a limited sample of the negative dipstick slides were assessed microscopically as it was logistically impossible to test all of them. The

results of the present study indicate that in the study population the OptiMAL® dipstick was able to identify more than 90% of pregnant women with malaria infection and correctly labelled about 85% non parasitaemic pregnant women. The high positive and negative predictive values and the corresponding likelihood ratios of a positive or negative test indicate that not many pregnant women were incorrectly diagnosed. However, the test performed poorly if parasite densities were below 50/μL.

Few studies have reported the use of rapid diagnostic tests in pregnant women but mainly for the purpose of diagnosing placental malaria at delivery (Leke *et al.* 1999; Mankhambo *et al.* 2002; Singer *et al.* 2004). All these studies reported the tests as having good diagnostic features apart from one (Mankhambo *et al.* 2002), which doubts the sensitivity of the OptiMAL® test to adequately diagnose placental malaria. A recently published study reporting the of use OptiMAL® dipsticks in the diagnosis of malaria in pregnant women attending antenatal clinics in Nigeria (VanderJagt *et al.* 2005) found the test to be insensitive compared to microscopy and PCR. However, it is not possible to compare this result to our study because the authors did not report the sensitivity, specificity, predictive values or likelihood ratios.

The OptiMAL® dipstick test is expected to produce similar results in all environments if the test is conducted according to the manufacturer's instructions. However, in our experience during the dry months of the year, the wash well dried up too soon if the manufacturer's instruction of filling this well at the beginning of the test process was followed. Under these conditions, the optimum results were obtained when the wash well was filled just before the dipstick was transferred into it.

The screening process with the OptiMAL® dipsticks fitted well within the routines of the antenatal clinic schedule. It did not cause any disruptions to the flow of work at the clinic throughout the study period. The high cost of RDT kits ranging between (US$1.00 to US$3.50)

per test compared to the cost (US$0.40) of using microscopy (Hanson *et al.* 2004) coupled with the relatively cheap and sensitive CQ and SP supported the argument that RDT use in moderate to high transmission areas may not be cost effective (Wongsrichanalai 2001; Fernando *et al.* 2004; Hanson *et al.* 2004). However, in Ghana the average cost of microscopic diagnosis of malaria are 10,000 and 15,000 cedis in public and mission hospitals respectively. At an exchange rate of US$1.00 to 9,120 Ghanaian cedis, the cost of microscopy per test is more than US$1.00, which was the cost of the OptiMAL® dipstick per test used in the present study. So cost may no longer be an issue limiting the wider use of RDTs in routine health care in Ghana. RDTs not being quantitative is a limiting factor (Wongsrichanalai 2001) but since peripheral parasite density in pregnancy may not correlate with placental parasitaemia, the use of OptiMAL® dipsticks to detect circulating pLDH may be of public health significance. Antenatal RDT screening and treatment of only those with positive results would mean that: -

i. Therapeutic judgements would be based on accurate diagnosis and so minimise wrongful antimalarial drugs prescription. The reduction of the excess and unnecessary antimalarial drugs prescription and use would save cost to offset the cost of using rapid diagnostic tests (Hanson *et al.* 2004).
ii. Accurate diagnosis and rational drug use are most likely to reduce drug pressure, which is implicated in the spread of parasite resistance to antimalarial drugs (Wernsdorfer 1994; Wongsrichanalai 2001).
iii. Pregnant women would benefit from early diagnosis and treatment of antenatal malaria which is likely to reduce the complications associated with malaria infection in pregnancy.
iv. Exposure of pregnant women to the newer and more expensive antimalarial treatment options being recommended for national policies but whose safety remains largely untested in pregnant women will minimise.

8.4 Clinical presentation of malaria in the study women

Fifty-eight percent of the women admitted to not being well in the last 5 days prior to enrolment and headache, fever, general malaise, dizziness and easy fatigability were their main complaints. However, only 7% of them had an axillary temperature of 37.5°C or above at enrolment. The symptoms were associated significantly with higher baseline peripheral parasite density. The symptoms were solicited from the women and so their responses depended on their understanding of the questions posed to them and their recall of the occurrence of the symptoms. To reduce any bias this limitation might introduce, the same set of questions were posed to all the study women who were parasitaemic and a control group of non-parasitaemic pregnant women, and the time of recall limited to the last five days prior to screening and enrolment. In the control group of pregnant women without parasitaemia, the proportions of ill health including fever, headache, vomiting, general malaise, tiredness and dizziness were significantly lower than in those with parasitaemia.

In stable malaria transmission areas, malaria in pregnancy is known to be frequently asymptomatic with maternal anaemia and low birth weight being the main features (Brabin 1983; Brabin 1985; Verhoeff *et al.* 1999; Shulman *et al.* 2001; Steketee *et al.* 2001; WHO 2004). McDermott and others reported that none of 19 Malawian pregnant women in their study who had parasitaemia at enrolment had a history of fever or symptoms related to malaria (McDermott *et al.* 1988). Similarly, Steketee and others did not observe any difference in the frequency of history of fever between parasitaemic and non-parasitaemic Malawian pregnant women (Steketee *et al.* 1996). In our study population malaria in pregnancy is often symptomatic. This is new and contrary to what is known to be the clinical manifestations of malaria in pregnancy in areas with stable malaria transmission such as our study area. These symptoms mimic pregnancy related symptoms but because they are associated significantly with parasite density, they must be

seriously considered. Possible explanations as to why this has not been noted earlier are given below.

The first explanation is that, services provided at antenatal clinics do not include screening for malaria infection in pregnancy although the clinics are used to promote malaria control measures. A review of the antenatal records and the record capture forms used at the clinic in Nkoranza suggests that the only screening (clinical or laboratory) that goes on at the clinic is aimed at detecting pre-eclampsia and anaemia. This is the practice in the rest of Ghana (GSS/NMIMR/ORC 2004). The midwives monitor blood pressure and test for protein in the urine to help detect pre-eclampsia, estimate haemoglobin to help detect anaemia and prescribe haematinics and malaria chemoprophylaxis. Symptoms are only noted at antenatal clinics when the pregnant woman reported them. The midwives, for example, are able to make a connection between headache, high blood pressure and urine protein to mean pre-eclampsia; and dizziness, low Hb concentration and obvious pallor to mean anaemia. Although pregnant women may give complaints similar to those in this study no possible connection is made between the symptoms and malaria. Malaria chemoprophylaxis and haematinics are prescribed only as a routine practice at the antenatal clinics.

Secondly, the presenting symptoms may be mild, unspecific, and self limiting which is typical of adult malaria (WHO 2000a). The pregnant woman may not report them if not asked. In the present study, the symptoms were solicited.

8.5 The liver and malaria during pregnancy

Pregnancy rarely causes alterations in the levels of AST, ALT, GGT or bilirubin (Knox and Olans 1996; Riely 1999). Thus, any elevation in these parameters is abnormal. In the present study, these parameters were raised above normal in a proportion of study women at enrolment. The differential diagnosis of these apparent abnormal tests at baseline is not clear. It could have resulted from defects in sampling which could cause samples to haemolyse or defects in methods used to assess these parameters but the consistent pattern seen make this possibility very unlikely. Liver disease conditions in pregnancy that may cause abnormal laboratory tests include hyperemesis gravidarum, cholestasis of pregnancy, acute fatty liver of pregnancy (AFLP), HELLP (haemolysis, elevated liver tests and low platelet) syndrome, drug induced hepatoxicity, preeclampsia and eclampsia (Knox and Olans 1996; Riely 1999). The clinical assessments at enrolment did not suggest any of the above conditions. Apart from drug toxicity and probably viral hepatitis, these conditions are related to gestational age. However, in the present study, gestational age did not have a significant association with the abnormal laboratory tests. Blood pressures for all the study women were normal at enrolment and remained so throughout the day 28 follow up period. Platelet counts were not assessed in this study nor was it determined if there were pre-existing liver diseases by other means such as ultrasonography. However, it is known that the prevalence of hepatitis C virus infection is very high in some populations in Ghana (Wansbrough Jones *et al.* 1998).

Severe malaria in adults has been associated with hepatic dysfunction with increased AST, ALT, GGT and total bilirubin concentrations and may indicate poor prognosis (WHO 2000a). It is most likely that malaria parasites were responsible for the abnormal liver function tests at enrolment in the present study. The argument for this is that a control group of non-parasitaemic pregnant women who donated blood samples for liver functions tests rarely had elevated liver enzymes or bilirubin. Secondly, following treatment, total bilirubin and its fractions

returned to normal. Thus malaria in pregnancy although it may present with mild unspecific symptoms is actually dangerous and may have fatal consequences.

8.6 Treatment efficacy

Treatment efficacy in this study expressed as parasitological failure and haematological recovery at day 28 after starting treatment was assessed along the lines of the WHO protocol for monitoring antimalarial drug-resistance (WHO 2001a). However, responses to treatment in clinically ill children, for whom the WHO protocol was designed, may be different from adult pregnant women who are often asymptomatic. And so the emphasis in this study was on monitoring parasitological response following treatment. Women were not followed up on days one and two after initial treatment to supervise the second and third doses of the treatment. This means that adherence to the treatment regimes may not have been 100% but it was expected that it would be higher than it would be in the routine health care delivery system. Colour coding and pre-packaging increased the chances that a full course of treatment would be taken appropriately. Thus within the spectrum of an efficacy or effectiveness study, this one is nearer to the efficacy end of the spectrum. Ninety-three percent and 94% of all randomised study women completed the day 28 and day 14 follow ups respectively and so the study sample size retained its power to detect any differences in proportions of parasitological failure between CQ and the test drugs. The individual randomisation and the double blind design of the study ensured that confounding and selection bias was substantially limited if not totally eliminated.

8.6.1 Parasitological outcomes

The study showed that the proportion of treatment failures in pregnant women was low. AQ alone and its combination with SP performed better than to CQ in clearing peripheral parasitaemia at both days 14 and 28. The parasite prevalence corrected for new infections by msp-2 genotyping was 0% at day 14 in the AQ+SP combination group and one percent at day 28. The results of 33 samples could not be determined by PCR genotyping. In 21 samples the PCR failed in the follow up day pairs, in 4 samples it failed in the day 0 pairs and in 7 samples it failed in both day 0 and follow up day pairs. The reasons could be due to suboptimal storage conditions of the filter paper samples, or ineffective DNA extraction. However, it is also known that parasite genotypes detected in one sample may not often be present in a matching sample in pregnant women suggesting a differential sequestration of different genotypes (Kamwendo *et al.* 2002). It has been found in children (Bruce *et al.* 2000) and in pregnant women (Kamwendo *et al.* 2002) that an infection is composed of different parasite genotypes such that at any time some genotypes may be sequestrated and others circulating. However in the present study we took only one sample per women on day 0 and on follow up days and so one can only speculate that the above explanations account partly for our findings.

We are unaware of any previous publication reporting the efficacy of AQ or its combination with SP in the treatment of malaria infection in pregnancy. However, the efficacy of AQ alone or its combination with SP or artesunate against CQ has been studied in children. In Ghana, recent studies in children have shown that AQ or its combination with SP or artesunate were better at clearing parasitaemia compared to chloroquine (Mockenhaupt *et al.* 2005; Oduro *et al.* 2005). Similar studies in children in other places have confirmed the superiority of AQ alone or in combination with SP or artesunate over CQ (Brasseur *et al.* 1999; Staedke *et al.* 2001; Basco *et al.* 2002; Dorsey *et al.* 2002; Abacassamo *et al.* 2004). Olliaro and colleagues in a systematic review in

1996 (Olliaro *et al.* 1996) compared the efficacy of AQ with CQ or SP. Their review showed that AQ was superior to CQ at clearing peripheral parasitaemia at day 7 and 14 post treatment while SP was superior to AQ at clearing parasitaemia at days 14 and 28 post treatment.

As expected, the combination of AQ and SP performed best suggesting that this combination could be a useful option in the treatment of malaria in pregnant women in other parts of Ghana or in other areas where the antimalarial drug resistance levels and malaria transmission conditions are similar to those of the study area. This would not be the case in areas where resistance to CQ, AQ or SP is high like in East Africa and south-east Asia.

Parasitological failure by day 28 occurred most frequently in those with high baseline parasite densities (1000/μL or more), Hb below 11g/dl and prior ill health; and in the primigravidae, nulliparae and primiparae. Nulliparous, primigravid women had the highest risk of parasitological failure. This agrees with the findings reported by Keuter and others in Kenya (Keuter *et al.* 1990). However, high baseline parasite density was not a predictor of treatment response in women who received the AQ+SP combination because there was only 1% true failure.

8.6.2 Haematological response

There was a general and progressive improvement in Hb concentrations during the follow up with a decrease in the proportions of anaemic pregnant women in all the treatment groups. These changes in haemoglobin were not related to the baseline parasite density, baseline Hb, parity, gravidity or age. AQ alone or in combination with SP was associated with higher increases in Hb at day 28 follow up after the start of treatment compared to CQ and SP. Similar improvements in haemoglobin concentrations have been observed in studies in which antimalarial drugs were used either for prophylaxis (Spencer *et al.* 1987; Greenwood *et al.* 1989;

Cot *et al.* 1998) or IPT (Parise *et al.* 1998; Shulman *et al.* 1999) or treatment (Steketee *et al.* 1987; Keuter *et al.* 1990; McGready *et al.* 2000; McGready *et al.* 2001; McGready *et al.* 2005) in pregnant. The increase in haemoglobin concentrations correlated with the decrease in parasite prevalence seen at follow up suggesting that falciparum parasitaemia was responsible for the anaemia in the pregnant women. Also associated with the increases in haemoglobin concentration in the present study, were significant decreases in the concentrations of total bilirubin and its fractions suggesting that the antimalarials had stopped the haemolysis. Therefore post treatment monitoring of haemoglobin concentrations can be used to indicate clinical efficacy of antimalarial treatment in pregnant women. Iron and folate supplementation and the treatment of hookworm infestation in those infested may also contribute to the improvements in haemoglobin concentrations as shown by (Shulman *et al.* 1996; Dreyfuss *et al.* 2000). Their contributions could not be assessed in the present study as all women received iron and folate supplements but they are known to be helpful for improving anaemia in pregnant women (Fleming 1989; Menendez *et al.* 1994; Msolla and Kinabo 1997; Verhoeff *et al.* 1998; Huddle *et al.* 1999; Mockenhaupt *et al.* 2000).

8.7 Safety and tolerance

The safety of antimalarial drugs used either for chemoprophylaxis, IPT or case management in the control of malaria in pregnancy has been the subject of debate and concern over the years. The incidence of symptoms and signs, and laboratory indicators associated with the test drugs reported in the present study are discussed in the following sections of this chapter.

8.7.1 Symptoms and signs

Seventy-five percent of women at day 3 follow up reported an adverse effect. General weakness, dizziness, vomiting, itching and nausea were the 5 most frequent adverse effects reported by the women. As expected, women who took SP had the least number of complaints while about 90% of the complaints came from women in the AQ and the AQ+SP combination groups. Notably the reports of weakness, vomiting, dizziness and nausea were highest in the AQ and AQ+SP groups and least in the SP groups. These complaints were not associated with age, baseline Hb, baseline parasite density, parity, gestation or prior ill health. The incidence of these complaints was higher than those reported by pregnant women who were non-parasitaemic and were not treated. Therefore it is most probable that the high incidence of complaints on day 3 was caused by the test drugs. Because many of the adverse effects noted are similar to the symptoms caused by pregnancy itself, the inclusion of a control group of non-infected pregnant women, a novel feature of this study, was very helpful in ascertaining the proportion of complaints that could be attributed to the drugs. Despite the symptoms related to treatment with AQ or AQ+SP, compliance was high. More than 90% of the study women completed their day 3 follow up. About five percent of the study women did not finish their full course of treatment due to the adverse effects. About 32% of those who reported adverse effects claimed that performance of their routine household chores had been inhibited after the start of treatment; compared to about 24% of untreated non-parasitaemic control group who also claimed that they could not perform their household chores on day 3 after screening. It is possible that the study women tolerated the adverse effects and complied with the treatment because they understood that they had malaria and were aware of what might happen if they did not comply. Women in the present study probably felt involved when they saw the positive reaction bands of the OptiMAL® dipsticks and understood what they meant. Compliance with AQ or AQ+SP might not be so good if these drugs were used for IPTp. Olliaro and colleagues in their review of trials involving

the use of AQ, SP or CQ for prophylaxis or treatment reported similar adverse effects as in this study but did not find any significant differences in incidence among the treatment groups (Olliaro *et al.* 1996).

Two cases of non-life threatening skin rash were thought to be probably related to the test drugs. They were reported on day 7 after the start of treatment and disappeared within a week. One woman took SP alone and the other took the AQ+SP combination. No other conditions were associated with the rash and the women did not receive any specific interventions. SP is known to be associated with rare but severe cutaneous reactions which could be life threatening such as Stevens-Johnson syndrome (Phillips-Howard and West 1990; Steffen *et al.* 1990; Luzzi and Peto 1993; Sturchler *et al.* 1993) but the lesions seen in this study were not of this type. Reactions similar to the one reported in this study were reported by David and others in Sierra Leone in children who took Maloprim for prophylaxis fortnightly for 3 or more months (David *et al.* 1997).

8.7.2 Liver enzymes

There was a general rise in AST activity at day 14 particularly in the AQ group compared to baseline activities. The mean AST levels returned to baseline levels at day 28. This is similar to what was found by Sturchler and others in travellers who used AQ, SP or CQ for prophylaxis. They found an association between AQ and male SP users and increased AST activity but which was significant only in the AQ group (Sturchler *et al.* 1987). The rise in AST activity in the present study could be considered as a transient derangement associated with the antimalarials. They were not associated with any clinical manifestations of hepatitis.

The more liver-specific ALT showed a similar pattern but contrary to expectation the mean ALT levels in the AQ group decreased steadily throughout the follow up period to below baseline levels at day 28.

The pattern of change in the GGT was different. The mean GGT values increased throughout the follow up period in all the treatment groups but were all within the normal range. It is not clear what was responsible for this pattern. Although GGT cannot help in discriminating between liver diseases, it is considered the most sensitive enzymatic indicator of liver disease; high values are rare in the absence of liver disease (Riely 1999). GGT may be elevated in response to toxic effects of drugs on liver cells (Lee 2003). It may also be raised in patients with liver cancer, heavy alcohol drinkers or patients with alcohol cirrhosis and in patients receiving anticonvulsants such as phenytoin or phenobarbital (Knox and Olans 1996; Riely 1999; Lee 2003). However, these conditions cannot explain the consistent pattern of increase in all the treatment groups throughout the follow up. It seems plausible that all the drugs used in the present study had some effect on the hepatobiliary system to cause elevations in GGT. Interactions of the test drugs with other unprescribed agents like drugs or herbal concoctions may also be responsible. Pregnancy itself increases the risk of drug induced hepatoxicity.

In healthy, non pregnant expatriate workers and their families in Nigeria, who were receiving long term prophylaxis with mefloquine plus SP combination or CQ, Kollaritsch and others observed irregular deviations of AST, ALT and GGT levels from normal levels at baseline and during chemoprophylaxis and so did not relate them to the drugs (Kollaritsch *et al.* 1988). In a female volunteer, they observed a six-fold increase in GGT levels at the end of an 18 months follow up period. They attributed the abnormal levels in AST, ALT and GGT to chronic alcoholism in some of the volunteers.

8.7.3 Bilirubin

Bilirubin levels declined after the start of treatment to below baseline levels at both day 14 and day 28. The possible explanation for this is cessation of the haemolysis caused by the malaria parasites as indicated in section 8.6.2. Also it is the only biochemical test that was significantly associated with Hb and parasite density at enrolment.

8.7.4 White blood cells

Leucopoenia is the most feared adverse effect from AQ treatment. Earlier studies have associated the prophylactic use of AQ to leucopoenia due to neutropaenia (Hatton *et al.* 1986; Neftel *et al.* 1986; Rhodes *et al.* 1986; Phillips-Howard and West 1990) or to lymphopoenia (Sturchler *et al.* 1987). Sturchler and others did not find a significant difference in the degree of leucopoenia caused by AQ, SP or CQ (Sturchler *et al.* 1987). In the present study, the proportions of neutrophils increased after the start of treatment without any significant differences in the treatment groups during the follow up period. The same pattern was shown when all granulocytes (neutrophils, basophils and eosinophils) were considered, apparently because the neutrophils form about 80% of the granulocyte population. There was a non significant decrease in the overall average lymphocyte count on day 3 follow up compared to baseline counts. On days 7, 14 and 28 the overall mean decreases in lymphocyte counts relative to baseline counts were significant but unrelated to the type of treatment received. The mean total white cell count decreased significantly on day 3 but unrelated to the type of treatment received. Subsequently, there were overall mean increases in the total white cell counts but again not related to the type of treatment received. The present study did not find any significant differences in the post treatment total and differential white cell counts of the treatment groups as did earlier studies. Apart from the post treatment lymphopoenia noted, there was no decrease

in the total white cell counts and neutrophils but rather an increase. Possible explanations for this are that total white cell counts are generally increased in pregnancy and although malaria is a cause of leucopoenia (Mckenzie *et al.* 2005) severe malaria is also associated with leucocytosis (Modiano *et al.* 2001). The mean decrease on day 3 was probably due to the combined effects of the drugs and the parasite loads. Besides, the earlier findings were in those who used amodiaquine for prophylaxes and so barring any differences in gender, age, race and methodology, a clear pattern may not emerge after a one off treatment dose.

8.8 Pregnancy outcome

The parasitological and haematological outcomes at delivery and at six weeks postpartum, incidence of low birth weight and the incidence of adverse neonatal outcomes are discussed in the following sections of this chapter. The analyses of pregnancy outcomes are limited by the large numbers of women who were lost to follow up at delivery and postpartum. Those lost to follow up at delivery migrated outside the study area before delivery to be with their parents or families. If we expected pregnancy outcome records on at least 90% of those who completed the day 28 follow up, then the data available for the analyses was only 50% for delivery Hb, 62% for parasite prevalence, 58% for birth weight and 70% for postpartum Hb and parasite prevalence analyses. Thus the power to detect any differences in treatment was reduced.

8.8.1 Parasitological outcomes

A placenta blood smear or impression smear was more sensitive in detecting parasitaemia at delivery than peripheral blood smear. Compared to CQ, the test drugs appeared to have some protective effect against peripheral parasitaemia but this was only significant in women who

received the AQ+SP combination therapy. Compared to CQ, SP appeared to have the most protective effect against placenta parasitaemia. Nulliparity and high baseline parasite density were both significantly associated with peripheral parasitaemia at delivery. Nulliparity alone was associated with placental parasitaemia detected by impression smear and not the placental blood smear whilst a high baseline parasite density was associated significantly with placental parasitaemia detected by placenta blood smear and not by placenta impression smear. Gestation at enrolment however, did not have an association with either peripheral or placenta parasitaemia at delivery.

At six weeks postpartum, the prevalence of peripheral parasitaemia was less than 5% indicating that a significant clearance of parasitaemia occurs after delivery. This finding is similar to the finding in some earlier studies (Ladner *et al.* 2002). A plausible reason for this is the fact that delivery removes the immunosupression and hence susceptibility to malaria (Nguyen-Dinh *et al.* 1988). On the contrary Steketee and others did not find any differences in parasite prevalence at antenatal, delivery and within 6 months of postpartum in both non-HIV and HIV Malawian women (Steketee *et al.* 1996) and Diagne and others found an increase in malaria episodes within 2 months postpartum in Senegalese women (Diagne *et al.* 2000).

8.8.2 Maternal haemoglobin at delivery

Nulliparity and teenage pregnancy were associated significantly with maternal Hb at delivery. The mean maternal Hb at delivery was higher than the mean during the day 28 follow up. Compared to CQ, the test drugs appeared to have some protective effect against maternal anaemia but this was significant only in study women who received the AQ+SP combination therapy. For study women who had retreatment prior to delivery, AQ and AQ+SP had very significant protective effects against maternal anaemia at delivery. This is expected because as discussed in section

8.6.2, antimalarial drug interventions either for prophylaxis or treatment and iron and folate supplementation, lead to improvements in maternal Hb concentrations.

At 6 weeks postpartum, only two of the study women had an Hb below 8g/dl while more than 75% had Hb of 11g/dl or above. While the reduction in falciparum parasitaemia may partly explain this, an improvement in nutrition may also play a major part. There are unsubstantiated reports in the study area that pregnant women generally do not take the iron and folate supplementations to avoid having big babies and hence caesarean sections.

8.8.3 Birth weight

Nulliparity and maternal anaemia were associated significantly with a low birth weight. The results agree with evidence from other studies that showed that maternal anaemia and low parity are risk factors for low birth weight (McGregor *et al.* 1983; Kramer 1987; Brabin *et al.* 1990; Brabin and Piper 1997; Kasumba *et al.* 2000; Lone *et al.* 2004). Low parity and maternal anaemia are also known predictors of placenta parasitaemia and so it was expected that low birth weight would be associated with peripheral or placenta parasitaemia (Brabin *et al.* 1996; Brabin and Piper 1997; Brabin *et al.* 1999; Shulman *et al.* 2001; Brabin *et al.* 2003; Guyatt and Snow 2004) at delivery but in the present study neither peripheral nor placenta parasitaemia examined microscopically nor gestation at enrolment was associated with the incidence of low birth weight. It however, appears that the risk of low birth weight depends on how much malaria related damage has been caused in the placenta by previous infections (Kaushik *et al.* 1992; Menendez *et al.* 2000; Shulman *et al.* 2001). So an association between placenta parasitaemia and low birth weight in the study population is possible and might have been detected if placental biopsy had been done.

8.8.4 Adverse pregnancy outcomes

The rates of abortions, preterm deliveries and still births among the study women were no more than the rates for non study women who delivered at St Theresa's hospital. Among the study women, the type of treatment did not have any association with the incidence of these events. There were no congenital defects judged to have been caused by the study drugs. This does not disprove the fact that the study drugs may have teratogenic effects. It only confirms the fact that these serious adverse effects known to be associated with these drugs are indeed rare and that a larger sample size than in the present study would be needed to detect them. Earlier studies involving standard therapeutic doses of the study drugs did not show excess risk of adverse foetal outcomes over local rates (Wolfe and Cordero 1985; Steketee *et al.* 1987).

8.9 Implications for research

This study has shown that only 20 to 30% of pregnant women attending antenatal clinics had *P. falciparum* parasitaemia; screening and treatment improved haemoglobin concentrations in the women; AQ alone or its combination with SP was most efficacious and safe but least tolerable; SP was most tolerable. Although screening and treating mothers with slide positive malaria appears to be feasible and beneficial for the mother, the effect of this approach on placental malaria and birth weight compared to IPT is unknown. SP-IPT not only clears parasitaemia but also provides intermittent chemoprophylaxis. If treatment was offered only to women who were slide positive (up to 30%), the opportunity of providing intermittent chemoprophylaxis will be missed for the remaining 70% of women. On the other hand if AQ alone or its combination with SP was recommended for IPT, the minor adverse effects associated with it might reduce women's compliance to the regime. The current national policy of SP-IPT for pregnant women has been shown to be efficacious, easy to administer, safe and tolerable (Parise *et al.* 1998; Verhoeff *et al.* 1998; Shulman *et al.* 1999; Rogerson *et al.* 2000; Njagi *et al.* 2003; van Eijk *et al.* 2004; Kayentao *et al.* 2005). Currently, SP-IPT has been rated as having the most favourable effectiveness profile in terms of lower cost, compliance and reduced morbidity and mortality resulting from the prevention of maternal anaemia and low birth weight (Goodman *et al.* 2001; Newman *et al.* 2003). However, as coverage of the programme increases to cover all pregnant women and resistance to SP increases or when the number of doses has to be increased due to increased HIV prevalence then this rating of SP may change; and SP-IPT may not be the long-term approach to decreasing the risks posed by antenatal malaria to the mother and her baby.

If that happens, it is likely that SP would be replaced by AQ, AQ+SP or artesunate (AS) plus AQ combination (the current first line drug for uncomplicated malaria) for IPT. As the safety of AS+AQ combination for IPT in pregnant women is not proven it would be prudent to screen

women attending ANC using RDT and treat only those who are RDT positive. Even if AQ or AQ+SP are used for IPT the concern of tolerance and the low prevalence of parasitaemia (20-30%) warrant the need to consider the screening and treatment approach. However, there is no clear evidence to suggest that the screening and treatment approach is equally effective to reduce the adverse effects of malaria during pregnancy on mother and foetus. There is also some evidence that although ITNs and IPT are individually effective, the extra benefit of combining the two interventions is negligible (Njagi *et al.* 2003). The reduction in malaria related adverse pregnancy outcomes attributed to the large scale use of ITNs among Kenyan pregnant women are very encouraging (ter Kuile *et al.* 2003; ter Kuile *et al.* 2003). It has highlighted the need to address the issues of cost, retreatment and adequate and sustainable supply of bed nets to achieve the right impact from this intervention.

These scenarios pose the following questions which need to be addressed scientifically: -

i. What proportions of women who are RDT negative have placental malaria?
ii. What are the consequences of restricting antimalarial treatment to pregnant women who are RDT positive to the health of the mother and her baby?
iii. Is this "selective treatment" cost effective?
iv. Would ITN plus RDT screening and treatment, provide better protection for the pregnant women and foetuses?

These questions may be addressed by a three-arm study as follows: -

i. OptiMAL® antigen screening plus treatment of dipstick positives with AQ+AS (the current Ghanaian national policy) plus ITN
ii. SP-IPT for dipstick negatives plus ITN

The objective would be to compare the efficacy, safety and cost effectiveness of RDT screening and treatment with SP-IPT.

The objective would be to compare the efficacy, safety and cost effectiveness of RDT screening and treatment with SP-IPT.

Useful endpoints would be maternal Hb at 36 weeks of gestation; active or chronic placenta parasitaemia determined by histology, birth weight and the incidence of serious and non-serious adverse events.

8.10 Implications for policy and practice

Ghana's Ministry of Health currently recommends quinine as the first line drug of choice for treating malaria in pregnancy in the first trimester and, quinine or AS+AQ in the second and third trimesters. However, the seven-day regime of quinine and the adverse effects associated with it, might compromise adherence to the full course of quinine by both prescribers and pregnant women. Tinnitus and dizziness reported (McGready *et al.* 2001) to be frequently associated with quinine have the potential to make it intolerable (Fungladda *et al.* 1998). Quinine can also induce hyperinsulinaemia which potentially can aggravate hypoglycaemia (White *et al.* 1983; Looareesuwan *et al.* 1985; Adam *et al.* 2005) associated with malaria in pregnancy. These potential adverse events related to quinine are likely to discourage the use of quinine during pregnancy and thus a regime of quinine may be difficult to implement. A regime of AQ+AS combination also remains untested as there was no local data to support its efficacy and safety for treating pregnant women with malaria. The present study has shown that AQ or AQ+SP combination is efficacious and reasonably safe for treating malaria in pregnancy. This means that for the time being, quinine or artemisinin based combination therapies are not necessary for treating malaria in pregnancy in Ghana. However, recommending the AQ+SP combination for treating malaria in pregnant women is limited because of the likelihood of rising SP resistant *P. falciparum* as SP monotherapy is the current national policy for IPT during pregnancy. Thus the

AQ+SP combination may not provide a long term solution to the search of a safe and efficacious treatment of malaria in pregnancy (WHO 2003c). One option to prolong the use of the AQ+SP combination is to use the screening approach and offer AQ+SP combination therapy only to those women who have a positive dipstick test. However, this approach cannot be recommended until we know the answers to the questions mentioned in section 8.9. On the basis of the existing evidence, our recommendation would be to promote as much as possible the full package of interventions recommended by the WHO namely; intermittent presumptive treatment, case management plus the prevention and treatment of maternal anaemia and insecticide treated nets (WHO 2004). To maximise the impact of these interventions pregnant women need to have full access to all three interventions or combinations of them that are feasible and sustainable.

8.11 Conclusions

The present study has shown that malaria in pregnancy is often symptomatic in the study population. A screening programme using OptiMAL® dipsticks could detect parasitaemia in the pregnant women with high sensitivity and specificity during routine antenatal clinic sessions. Although the screening needed extra staff, it did not cause any disruption at all to work at the antenatal throughout the study period. The AQ+SP combination was the most efficacious regime in the treatment of parasitaemic pregnant women. It was safe and reasonably tolerated and could be used for the treatment of malaria in pregnancy. However, given the current policy of using quinine or AS+AQ for the treatment of malaria in pregnancy in Ghana, the AQ+SP combination as first line drug needs to be considered by countries that are now contemplating changing their national antimalarial drug policies.

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APPENDICES

APPENDICES

APPENDIX 1 INFORMATION SHEET AND CONSENT FORM

Title: Safety and efficacy of Amodiaquine and SP versus chloroquine in the treatment of malaria in pregnancy.

Investigators: Tagbor H, Chandramohan D, Browne E, Greenwood B.

The following was repeated in the local Akan language for the understanding of eligible pregnant women.

Malaria in pregnancy is life threatening to both the mother and the baby. In Ghana, chloroquine is the main drug we use for malaria treatment but it is no more effective for treating malaria, so more effective and safe drugs are needed urgently to treat malaria.

Amodiaquine (camoquine) which is similar to chloroquine in its action against malaria parasites was removed from the essential drugs list because of its association with some rare but serious adverse effects when taken more frequently. Its use has been recommended again for the treatment of malaria in combination with fansidar or artesunate. Studies from Tanzania and Kenya suggest that it is efficacious and safe when used in children. We in Ghana are contemplating changing the amodiaquine and its combination of SP or artesunate and want to test amodiaquine and fansidar against chloroquine. So we would like you to help us do this by participating in the study. The findings will help the Ministry of Health to better control of malaria in pregnancy.

If you agree to participate in this study, this is what it will involve.

1. We will ask you some questions about yourself including your general health, your education level and living conditions.

2. We will take a little blood by a prick of your fingertip to test for malaria and thinning of blood.
3. We will let you choose one of the 3 drugs by lottery.
4. If we find malaria parasites in your blood we will give you the drug you chose. You take the first dose here and the rest at home.
5. We will then visit you on the third and seventh day to find out how you are doing and take blood to test for malaria parasite and thinning of blood.

Every information we have on you will not be disclosed to anyone by us. Participation in this study is voluntary. If you do not wish to participate in the study, it will not affect the regular care you have been receiving from this hospital now or in the future.

Do you have any questions for us that will help you decide whether or not you want to take part in the study?

Do you understand what taking part in this study will mean for?

I certify that I have explained the above to _____ that she understood what I said and she has agreed to freely join the study.

Signature: Date: ____/____/_____

Name:

I have understood the explanation given to me by _____ and I agree to join the study.

Signature or Thumbprint: Date ____/____/_____

Name: _____

APPENDIX 2 SCREENING FORM ONE

NKORANZA ANTIMALARIAL DRUGS PROJECT
SCREENING FORM ONE

DATE: ________________ MIDWIFE'S NAME: ____________________

Screening number	Name	Village	ANC Number	Age	Gestation	Gravity	Parity	No. of children alive	No. of children dead	OptiMAL®	Included	Reason for exclusion (enter code)

APPENDIX 3 SCREENING FORM TWO

NKORANZA ANTIMALARIAL DRUGS PROJECT
SCREENING FORM TWO

Screening date: _____ Screening number _____

Name of eligible pregnant woman: _____

Age: _____

Date of birth: _____

Village/Location : _____

1. Her pregnancy is at least 16 weeks.	Yes	No
2. She has *P. falciparum* parasitaemia of any density with or without symptoms.	Yes	No
3. She is willing to participate and complete the test schedule, and has given informed consent.	Yes	No
4. She is willing to be delivered by a midwife in a health centre or hospital.	Yes	No
5. She lives within Nkoranza district.	Yes	No
6. She has had repeated stillbirths, eclampsia or other past bad obstetric history.	Yes	No
7. She has a history of severe adverse drug reactions to Septrin or Fansidar in the past	Yes	No
8. She has a haemoglobin level below 5.0 g/dl.	Yes	No
9. Has mixed infection	Yes	No
10. She has multiple pregnancy	Yes	No
11. She has malaria that is severe enough to require parenteral medication.	Yes	No

If responses to questions 1 - 5 are all YES, the PI/clinician or midwife **should invite the pregnant woman to enter the study**: If on the other hand a response to questions 6 - 11 is YES, the **woman should be given routine antenatal care as required by the national guidelines.**

APPENDIX 4 RECRUITMENT FORM ONE

NKORANZA ANTIMALARIAL DRUGS PROJECT
RECRUITMENT FORM ONE
IDENTIFICATION & BACKGROUND INFORMATION

1 Date of recruitment _____

2 Study ID number _____

5 First name (as on card) _____

3 Drug Code _____

4 ANC Card number _____

6 Last name (as on card) _____

7 Contact Person _____

8 Location _____

9 Landmarks _____

10 House number _____

11 Date of birth _____

12 Age (Years) ☐

13 Educational level reached ☐
1 None
2 Primary
3 JSS
4 SSS
5 Tertiary

14 Occupation ☐
1 Housewife
2 Farmer
3 Trader
4 Salary worker
5 Other (specify)

15 Religion ☐
1 Christianity
2 Islam
3 Traditional African Religion
4 None
5 Other (specify)

16 Ethnic Group ☐
1 Brong
2 Akan
3 Dagaba
4 Dagomba/Frafra/Kokomba
5 Ewe/Fante/Ga

17 Marital Status ☐
1 Married
2 Single

18 Socioeconomic Status
1 Access to portable water __Y/N__
2 Owns a farm __Y/N__
3 Owns radio __Y/N__
4 Owns TV __Y/N__
5 Owns bicycle/motorbike __Y/N__
6 Size of household ☐
7 Owns a house __Y/N__

19 Type of building ☐
1. Block/Brick
2. Mud
3. Thatch

20 Type of roofing ☐
1. Corrugated Roofing Sheets
2. Thatch

21 Type of floor ☐
1. Cemented
2. Uncemented

22 How many bed nets are there in your house? ☐

23 Do you use a bed net regularly? __Y/N__

APPENDIX 5 RECRUITMENT FORM TWO

NKORANZA ANTIMALARIAL DRUGS PROJECT
RECRUITMENT FORM TWO
CLINICAL ASSESSMENT

Study ID number _____ Drug Code _____

1 PAST MEDICAL HISTORY

Hypertension	__Y/N__
Diabetes	__Y/N__
Sickle cell disease	__Y/N__
Epilepsy	__Y/N__
Asthma	__Y/N__

2 PRESENT MEDICAL HISTORY

Sick in last 5 days?	__Y/N__
If yes OR no, please circle	
Fever & chills	Y / N / NS[vii]
Headache	Y / N / NS
Vomiting	Y / N / NS
General malaise	Y / N / NS
Cough	Y / N / NS
Dizziness	Y / N / NS
Painful micturition	Y / N / NS
Tired easily	Y / N / NS
General weakness	Y / N / NS
Waist pain	Y / N / NS
Lower abdominal pain	Y / N / NS

3 PHYSICAL EXAMINATION

Pallor	__Y/N__		SPLEEN
Axillary Temperature		1	Enlarged
Blood pressure		2	Not enlarged
Weight			LIVER
Height		1	Enlarged
Fundal height		2	Not enlarged
Foetal presentation			CVS
1. Cephalic		1	Normal
2. Breech		2	Abnormal
3. Transverse / oblique			
Foetal sound heard	__Y/N__		

[vii] NS = Not sure

APPENDIX 6 RECRUITMENT FORM THREE

NKORANZA ANTIMALARIAL DRUGS PROJECT
RECRUITMENT FORM THREE
OBSTETRIC ASSESSMENT

Study Identification Number _____ Drug Code _____

1 Gravidity [ ] 2 Parity [ ]

3 Last Menstrual Period _____ 4 Expected Delivery Date _____

5 **Events in previous pregnancies**

Bled during pregnancy	Y / N / NA
Sick & treated at hosp.	Y / N / NA
Hospitalised	Y / N / NA
Aborted pregnancy	Y / N / NA

No of children alive	[ ]
No of children dead	[ ]
If yes, when	[ ]

1. Died before birth
2. Pronounced dead after birth
3. Died before one month old
4. NA

6 **Previous Delivery** [ ]

1. Hospital Delivery
2. Normal
3. C/Section
4. Vacuum Extraction
5. Episiotomy
6. NA

Home Delivery [ ]

1. TBA
2. Self/Relative
3. NA

7 **Events in current pregnancy**

Bled or bleeding	__Y/N__
Sick & treated at Hosp.	__Y/N__
Hospitalised	__Y/N__

8 **Gestation in weeks by: -**

Woman	[ ]
Fundal height	[ ]
Ultrasound scan	[ ]
Foetal Presentation	[ ]

1. Cephalic
2. Breech
3. Transverse / oblique

Foetal Sound Heard __Y/N__

APPENDIX 7 RECRUITMENT FORM FOUR

NKORANZA ANTIMALARIAL DRUGS PROJECT
RECRUITMENT FORM FOUR
DRUG HISTORY

Study ID number _____

Drug Code _____

Known drug reactions

Dizziness	__Y/N__
Headache	__Y/N__
Sleeplessness	__Y/N__
Skin Rash	__Y/N__
Itchiness	__Y/N__
Palpitations	__Y/N__
Nausea	__Y/N__
Vomiting	__Y/N__
Jaundice	__Y/N__
Dark Urine	__Y/N__

Names of drugs allergic to (if known)

Septrin	__Y/N__
Aspirin	__Y/N__
Fansidar	__Y/N__
Chloroquine	__Y/N__
Other (Specify)	__Y/N__

Where did you get the drug

1. Health Facility
2. Chemical Shop
3 .Herbalist
4. Friend/Relative
5. NA

Have you taken chloroquine in last 2 months
__Y/N__

Have you taken fansidar in last 2 months
__Y/N__

APPENDIX 8 LABORATORY FORM ONE

NKORANZA ANTIMALARIAL DRUGS PROJECT
LABORATORY FORM ONE

DAY 0

Date _____ Screening Number _____

MICROSCOPY

PARASITE TYPE	PARASITE COUNT
P. F. TROPHOZOITES	
P. F. SCHIZONTS	
P. F. GAMETOCYTES	
P. M. TROPHOZOITES	
P. M. SCHIZONTS	
P. M. GAMETOCYTES	
P. O. TROPHOZOITS	
P. O. SCHIZONTS	
P. O. GAMETOCYTES	

BIOCHEMISTRY

TEST	RESULT
AST	
ALT	
GGT	
TOTAL BILIRUBIN	
DIRECT BILIRUBIN	
INDIRECT BILIRUBIN	

HAEMATOLOGY

TOTAL WBC	
NEUTROPHILS	
LYMPHOCYTES	
MONOCYTES	
EOSINOPHILS	
BASOPHILS	
HAEMOGLOBIN	

ELISA urine dipstick positivity

Chloroquine __Y/N__
SP __Y/N__
Both __Y/N__

Hookworm Infestation __Y/N__

Laboratory Technician/Microscopist

_____

APPENDIX 9 LABORATORY FORM TWO

NKORANZA ANTIMALARIAL DRUGS PROJECT
LABORATORY FORM TWO

DAY [3] [7] [14] [28]

Date _____ Study ID number _____

MICROSCOPY

PARASITE TYPE	PARASITE COUNT
P. F. TROPHOZOITES	
P. F. SCHIZONTS	
P. F. GAMETOCYTES	
P. M. TROPHOZOITES	
P. M. SCHIZONTS	
P. M. GAMETOCYTES	
P. O. TROPHOZOITS	
P. O. SCHIZONTS	
P. O. GAMETOCYTES	

BIOCHEMISTRY

TEST	RESULT
AST	
ALT	
GGT	
TOTAL BILIRUBIN	
DIRECT BILIRUBIN	
INDIRECT BILIRUBIN	

HAEMATOLOGY

TOTAL WBC	
NEUTROPHILS	
LYMPHOCYTES	
MONOCYTES	
EOSINOPHILS	
BASOPHILS	
HAEMOGLOBIN	

Laboratory Technician/Microscopist

_____

APPENDIX 10 FOLLOW UP FORM ONE

NKORANZA ANTIMALARIAL DRUGS PROJECT
FOLLOW-UP FORM ONE
DAY 3 FOLLOW - UP

Date **Study ID number** **Drug Code**

_____ _____ _____

ASSESSMENT OF ADVERSE EVENTS

Ask: Did you finish taking all the drugs? ___Y/N___

If no, why.

1. Adverse effects
2. Forgot To Take
3. N/A

Ask: Did you experience any problems after taking the drugs? __Y/N__
Whether yes OR no, enquire about the following and tick "Yes" OR "No" against the mentioned experience

REPORTED EVENTS	RESPONSE
Dizziness	__Y/N__
Headache	__Y/N__
Sleeplessness	__Y/N__
Skin Rash	__Y/N__
Itchiness	__Y/N__
Palpitations	__Y/N__
General Weakness	__Y/N__
Nausea	__Y/N__
Vomiting	__Y/N__
Sore Mouth	__Y/N__
Painful Swallowing	__Y/N__
Fever	__Y/N__
Dark Urine	__Y/N__
Vaginal Bleeding	__Y/N__
Other (specify)	__Y/N__

Able to perform routine activities

Yes
No

Field team's assessment

Axillary Temperature
Pallor __Y/N__
Jaundice __Y/N__

OUTCOME

1. Continue
2. Need Escape Medication
3. Delivered
4. Travelled
5. Missed her
6. Consent Withdrawn

_____

_____

_____

_____

_____

_____

Field Worker **Field Supervisor**

_____ _____

Verification by PI

_____

APPENDIX 11 FOLLOW UP FORM TWO

NKORANZA ANTIMALARIAL DRUGS PROJECT
FOLLOW-UP FORM TWO
DAY [7] [14] [28]

Date **Study ID number** **Drug Code**

_____ _____ _____

ASSESSMENT OF ADVERSE EVENTS

Ask: Did you experience any problems our last visit? __Y/N__
Whether yes OR no, enquire about the following and tick "Yes" OR "No" against the mentioned experience

REPORTED EVENTS	**RESPONSE**
Dizziness	__Y/N__
Headache	__Y/N__
Sleeplessness	__Y/N__
Skin Rash	__Y/N__
Itchiness	__Y/N__
Palpitations	__Y/N__
General Weakness	__Y/N__
Nausea	__Y/N__
Vomiting	__Y/N__
Sore Mouth	__Y/N__
Painful Swallowing	__Y/N__
Fever	__Y/N__
Dark Urine	__Y/N__
Vaginal Bleeding	__Y/N__
Other (specify)	__Y/N__

_____

_____

_____

_____

_____

_____

_____

Able to perform routine activities [ ]

Yes
No

Field team's assessment

Axillary Temperature [ ]
Pallor __Y/N__
Jaundice __Y/N__

OUTCOME [ ]

1. Continue
2. Need escape medication
3. Delivered
4. Travelled
5. Missed her
6. Consent withdrawn

Field Supervisor

_____

Field Worker

_____

Verification by PI

_____

APPENDIX 12 DELIVERY FORM

NKORANZA ANTIMALARIAL DRUGS PROJECT
DELIVERY FORM

Date of delivery _____ **Study ID number** _____ **Drug Code** _____

PREGNANCY OUTCOME

Maturity [ ]
1. Preterm Delivery (28-36 weeks)
2. Term Delivery (37-42 weeks)
3. Miscarriage (before 28 weeks)
4. Stillbirth (after 28 weeks)

Type of delivery [ ]
1. Normal S VD
2. Breech
3. Vacuum Extraction
4. C-Section

Reasons for surgical intervention [ ]
1. CPD
2. Poor Maternal Effort
3. Abnormal Presentation
4. Foetal Distress
5. APH
6. OTHER (SPECIFY) _____
7. Not Applicable

Placental Weight [ ]
Maternal Weight (before delivery) [ ]

Mother's condition in labour
Fever __Y/N__
Pallor __Y/N__
Jaundiced __Y/N__
Bleeding __Y/N__

Colour of liquor [ ]
1. Whitish
2. Yellowish
3. Greenish

Smell of liquor [ ]
1. Normal
2. Offensive

Date & time of onset of labour
_____

Date & time of end of 3rd stage labour
_____

Duration of labour
____hr. /____mins.

ASSESSMENT OF THE NEWBORN

APGAR Score at birth [ ]

Did baby need resuscitation? __Y/N__

If Yes, what is the duration of resuscitation? [ ]

Outcome of resuscitation [ ]
1. Successful
2. Floppy
3. Dead

Weight _____kg
Length _____cm
Head Circumference _____cm
Arm Circumference _____cm

Observed abnormalities [ ]
1. Face
2. Fingers
3. Arms
4. Legs
5. Toes
6. Body
7. Other (specify)
8. None

Is baby jaundiced?
__Y/N__

Midwife _____

Verification by PI / clinician _____

APPENDIX 13 DELIVERY LABORATORY FORM

NKORANZA ANTIMALARIAL DRUGS PROJECT
DELIVERY LABORATORY FORM

Delivery Date _____

Study ID number _____

Drug Code _____

Mother's Hb _____

Placental biopsy taken? ____YES/NO____

Mother's peripheral blood smear

PARASITE TYPE	PARASITE COUNT
P. F. TROPHOZOITES	
P. F. SCHIZONTS	
P. F. GAMETOCYTES	
P. M. TROPHOZOITES	
P. M. SCHIZONTS	
P. M. GAMETOCYTES	
P. O. TROPHOZOITS	
P. O. SCHIZONTS	
P. O. GAMETOCYTES	

Placental blood smear

PARASITE TYPE	PARASITE COUNT
P. F. TROPHOZOITES	
P. F. SCHIZONTS	
P. F. GAMETOCYTES	
P. M. TROPHOZOITES	
P. M. SCHIZONTS	
P. M. GAMETOCYTES	
P. O. TROPHOZOITS	
P. O. SCHIZONTS	
P. O. GAMETOCYTES	

Cord blood smear

PARASITE TYPE	PARASITE COUNT
P. F. TROPHOZOITES	
P. F. SCHIZONTS	
P. F. GAMETOCYTES	
P. M. TROPHOZOITES	
P. M. SCHIZONTS	
P. M. GAMETOCYTES	
P. O. TROPHOZOITS	
P. O. SCHIZONTS	
P. O. GAMETOCYTES	

Placental impression smear

PARASITE TYPE	PARASITE COUNT
P. F. TROPHOZOITES	
P. F. SCHIZONTS	
P. F. GAMETOCYTES	
P. M. TROPHOZOITES	
P. M. SCHIZONTS	
P. M. GAMETOCYTES	
P. O. TROPHOZOITS	
P. O. SCHIZONTS	
P. O. GAMETOCYTES	

Laboratory Technician/Microscopist

_____

APPENDIX 14 POSTPARTUM FORM

NKORANZA ANTIMALARIAL DRUGS PROJECT
POST PARTUM FOLLOW-UP FORM

Follow-up Date **Study ID number** **Drug Code**

_____ _____ _____

Enquire about health of baby ☐

1. Alive
2. Dead
3. Sick

Mother's Hb ☐

Peripheral Smear
(P. falciparum count) ☐

If the baby is dead,
Ask when OR how long ago he/she died?

____/____/_____/

Ask what happened before he/she died?

_____

_____

_____

_____

_____

Field Worker

_____

Field Supervisor

_____

Verification by PI

_____

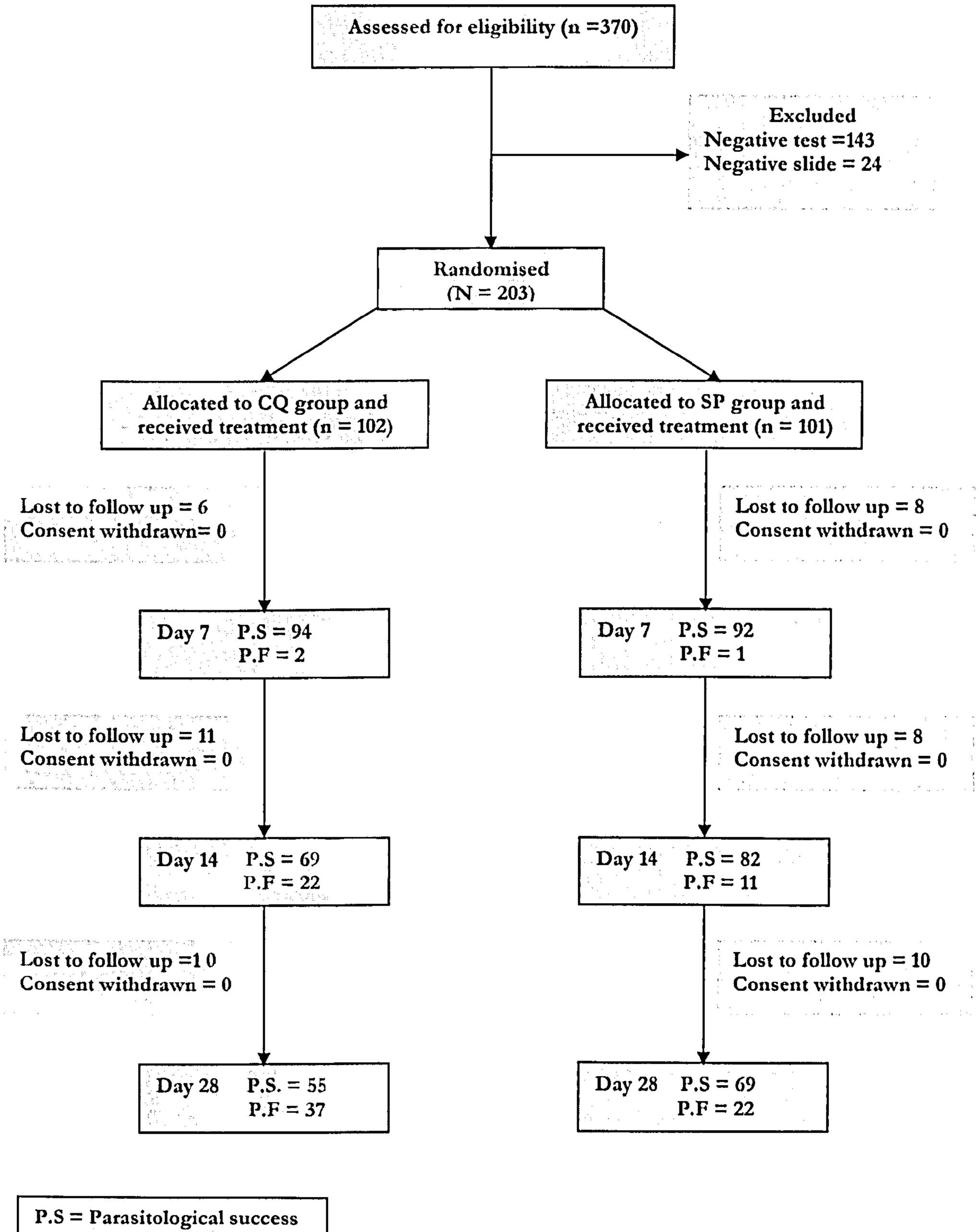
APPENDIX 15 Interpretation of microscopy results by microscopists from the St. Theresa's Hospital and Noguchi Memorial Institute of Medical Research

	Noguchi Memorial Institute of Medical Research														
	Day 0			Day 3			Day 7			Day 14			Day 28		
	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total
St. Theresa's Hospital															
Negative	128	0	128	86	0	86	48	0	48	75	0	75	52	0	52
Positive	13	106	119	1	3	4	1	0	1	1	4	5	6	20	26
Total	141	106	247	87	3	90	49	0	49	76	4	80	58	20	78
Agreement (%)	*94.7*			*98.9*			*97.9*			*98.8*			*92.3*		
Kappa Statistic	*14.1*			*8.17*						*7.95*			*7.33*		
p-value	*<0.001*			*<0.001*						*<0.001*			*<0.001*		

APPENDIX 16 Estimation of *P. falciparum* resistance to CQ and SP in children treated at St. Theresa's Hospital.

The rationale for this sub study has been given in section 3.13.3 of this thesis. A flow diagram of the screening process that led to randomisation and enrolment of children into the study is shown in **Figure 1.** A total of 370 children who were brought to the OPD of the St. Theresa's Hospital, were screened with OptiMAL® tests. Two hundred and three (54.9%) who had positive antigen test with microscopic confirmation of parasitaemia, were randomised to receive either CQ or SP. One hundred and forty-three who were OptiMAL® dipstick negative and 24 who had positive dipstick tests but negative microscopy were excluded.

Figure 1: - Diagram showing the flow of children through a 28-day follow-up period after treatment.



The baseline characteristics of the children and factors associated with parasite density at enrolment are shown in **Tables 1 and 2**. More than 98% of the children had a history of fever while 78% had a high temperature (37.5^{o} C or above) at enrolment as shown in **Table 1**. The mean age of the children was 2.2 years. There were no statistical differences between the groups with respect to baseline parasite density, age, history of fever or having a high temperature as shown in **Table 2**. Baseline parasite density was associated significantly with axillary temperature, history of fever and age.

Table 1: - Baseline characteristics of children under 5 according to treatment arm.

	SP		CQ		Total	
	n	(%)	n	(%)	n	(%)
Gender						
Female	50	(52.6)	53	(52.5)	103	(52.6)
Male	45	(47.4)	48	(47.5)	93	(47.4)
History of fever						
NO	2	(2)	2	(2)	4	(2)
YES	100	(98)	99	(98)	199	(98)
Temperature						
<37.5	27	(23.7)	23	(20.4)	50	(22)
37.5 & above	87	(76.3)	90	(79.6)	177	(78)
Mean (SD)	37.9	(0.9)	37.9	(0.9)	37.9	(0.9)
Parasite density/μL						
32 - 999	13	(12.9)	7	(6.9)	20	(9.9)
1000 & above	88	(87.1)	95	(93.1)	183	(90.1)
Geometric mean (95% CI)	12909	(8843 - 18844)	14534	(10008 - 21107)		
Range (min to max)	(32 - 520650)		(128 - 352000)			
Age						
< 3 years	49	(60.5)	53	(68)	102	(64.1)
3 to 5 years	32	(39.5)	25	(32)	57	(35.9)
Mean (SD)	2.3	(1.2)	2.1	(1.2)	2.2	(1.2)
Median (IQR)	2	(2)	2	(2)	2	(2)

Table 2: - Factors associated with baseline parasite density

	Baseline parasite density/μL						
	32 - 999		1000 & above		Total		p-value*
Temperature/ ^{O}C							
<37.5	8	(40)	33	(18)	41	(20.2)	
37.5 & above	12	(60)	150	(82)	162	(79.8)	
							0.04
History of fever							
No	2	10	2	(1.1)	4	(2)	
Yes	18	90	181	(98.9)	199	(98)	
							0.05
Age [years]							
< 3 years	10	(66.7)	47	(32.6)	57	(35.8)	
3 to 5 years	5	(33.3)	97	(67.4)	102	(64.2)	
							0.01
Gender							
Female	7	(36.8)	96	(54.2)	103	(52.6)	
Male	12	(63.2)	81	(45.8)	93	(47.4)	
							0.2

* Fisher's exact test

As shown in **Table 3 and Figure 2**, the parasitological failure by day 14 was 11.8% and 24.2% respectively in the SP and CQ groups. The parasitological failures by day 28 were 24.2% and 40.2% respectively for the SP and CQ groups. A comparison of the parasitological failure by day 14 and at day 28 in pregnant women of the main study and the children is shown in **Figure 3**. In both pregnant women and children SP performed better than CQ at clearing parasitaemia by day 14 and 28. However, the proportions of parasitological failure in pregnant women in both treatment arms were less than half the proportions in children at day 14 and about 75% of those in children by day 28.

Table 3: - Parasite prevalence at days 14 and 28 after the start of treatment in children under 5 years

	Parasite prevalence			Unadjusted		p-value	Adjusted *		p-value
	N	n	(%)	odds ratio (95% CI)			odds ratio (95% CI)		
DAY 14									
SP	93	11	(11.8)	1.0					
CQ	91	22	(24.2)	2.4	(1.1 - 5.2)	0.03	1.4	(0.6 - 3.5)	0.5
DAY 28									
SP	91	22	(24.2)	1.0					
CQ	92	37	(40.2)	2.1	(1.1 - 4.0)	0.02	1.3	(0.6 - 2.6)	0.6

* Odds ratios adjusted for gender, age and baseline parasite density

Figure 2: - Parasitological outcomes in children under 5 years determined by microscopy

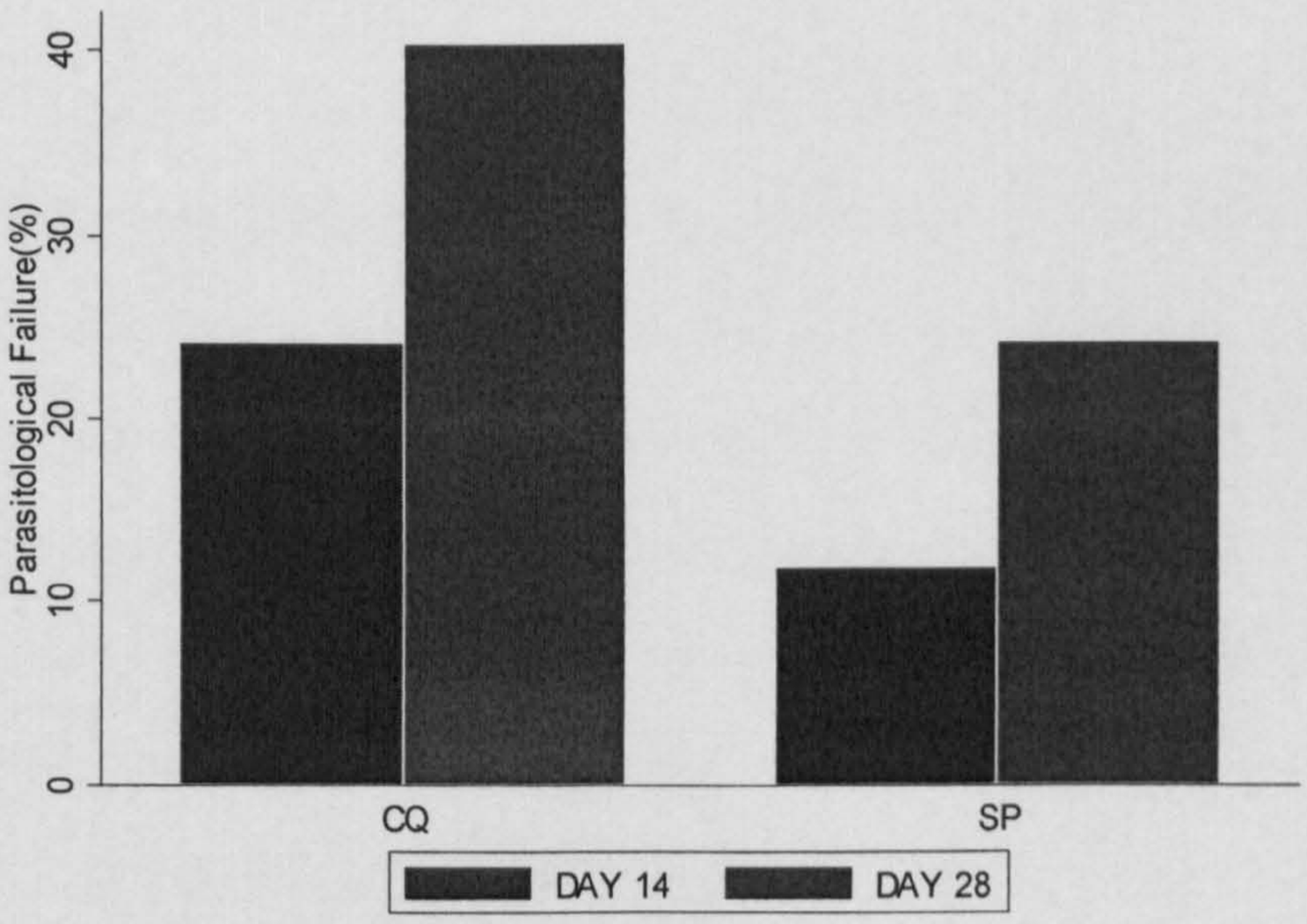
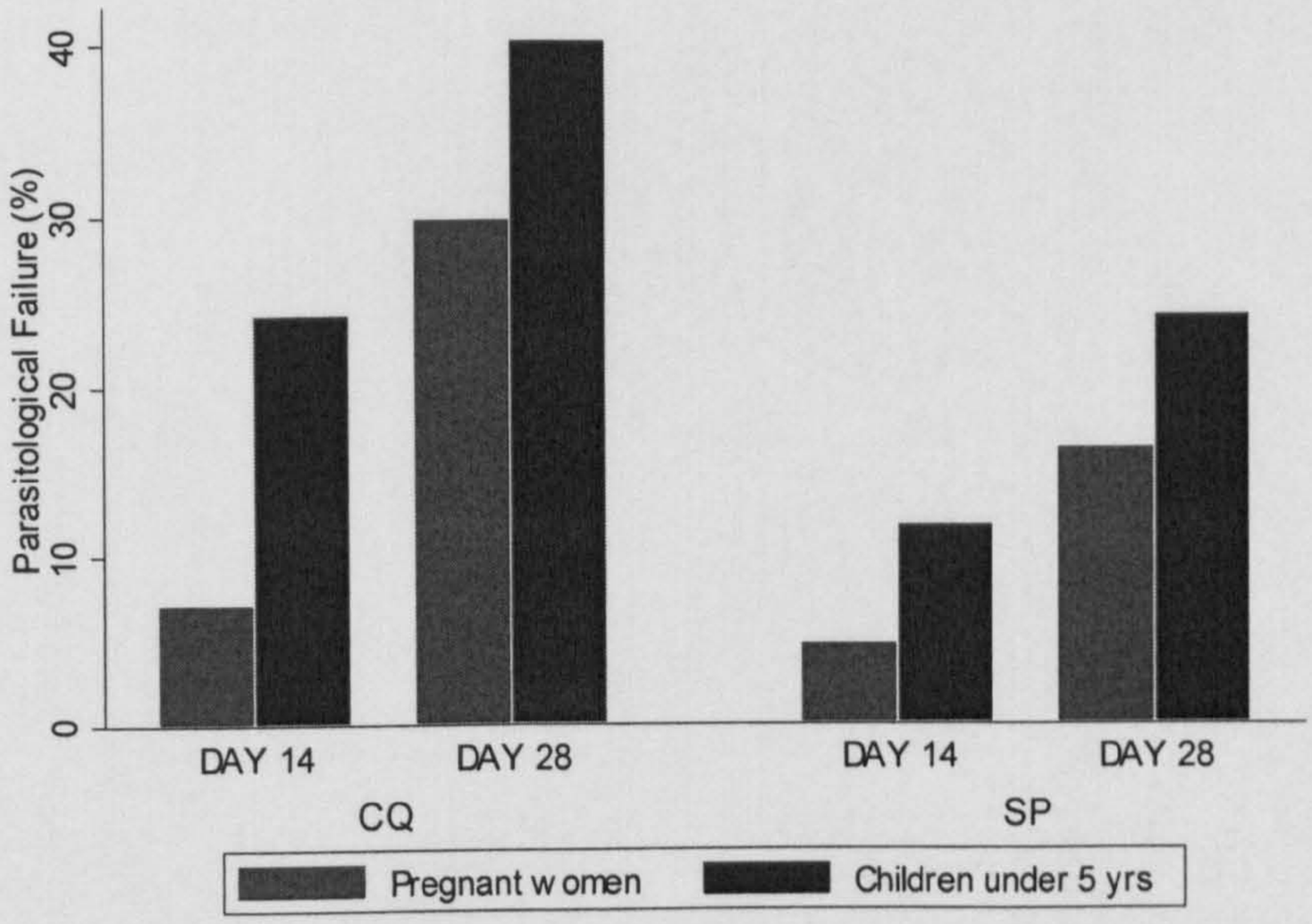


Figure 2: - Parasitological failure in pregnant women and children under 5 years determined by microscopy



The apparent differences would have been easier to explain if the parasitological failures in children were corrected for re-infections. The high parasitological failure in children compared to pregnant women may be due to the fact that 90.1% of the children compared to 46.8% of pregnant women had parasite densities equal to 1000/μL or above at enrolment. The differences in parasite at enrolment may the result of low level of acquired immunity to *P. falciparum* infection in children compared to pregnant women. High parasite burden together with low level of acquired immunity have been shown to increase the likelihood of parasitological failures (Djimde *et al.* 2003; Ittarat *et al.* 2003; Mayxay *et al.* 2003). And so although the drugs may be ineffective in children, these differences (White 2002) may explain why they performed relatively better in the pregnant women. It appears that the parasitological failure rates observed in children in classical drug resistance studies may not be applicable to pregnant women. However, in both groups they are high enough to warrant a change in treatment policy for more efficacious antimalarial treatment regimes.

APPENDIX 17 Liver enzyme activities and bilirubin concentrations of non-parasitaemic and parasitaemic pregnant women at enrolment.

	Non-parasitaemic		Parasitaemic	
	n	(%)	n	(%)
AST (U/L)				
Up to 12	181	(90.5)	514	(59)
12 to 24	19	(9.5)	262	(30.1)
>= 25	NA*	NA	95	(10.9)
Mean (SD)	8.1	(2.3)	13.9	(10.8)
Median (IQR)	7	(0)	10	(9)
ALT (U/L)				
Up to 12	200	(100)	755	(86.8)
12 to 24	NA	NA	77	(8.8)
>= 25	NA	NA	38	(4.4)
Mean (SD)	4.1	(0.7)	7.5	(7.8)
Median (IQR)	4	(0)	4	(4)
GGT (U/L)				
Up to 50	199	(99.5)	855	(99.5)
51 to 100	1	(0.5)	3	(0.4)
> 100	NA	NA	1	(0.1)
Mean (SD)	9.1	(9.5)	9.9	(9.3)
Median (IQR)	5	(8)	8	(7)
Total bilirubin (µmol/L)				
Up to 17.1	195	(97.5)	713	(81.9)
17.2 to 34.4	5	(2.5)	117	(13.4)
>= 34.5	NA	NA	41	(4.7)
Mean (SD)	6.3	(3.8)	12.1	(13.4)
Median (IQR)	5	(3)	8	(9)
Direct bilirubin (µmol/L)				
Up to 3.4	185	(92.5)	506	(58.1)
3.5 to 6.8	12	(6)	205	(23.5)
>= 6.9	3	(1.5)	160	(18.4)
Mean (SD)	2.0	(1.3)	4.9	(5.9)
Median (IQR)	2	(1)	3	(4)
Indirect bilirubin (µmol/L)				
Up to 13.7	198	(99)	775	(89)
13.8 to 27.4	2	(1)	77	(8.8)
>= 27.5	NA	NA	19	(2.2)
Mean (SD)	4.3	(3.1)	7.2	(8.7)
Median (IQR)	3	(3)	5	(6)

*Nobody in these categories