ATOVAQUONE-PROGUANIL COMBINATION FOR MALARIA TREATMENT: A SYSTEMATIC REVIEW WITH META-ANALYSIS

Abraham Rexford Oduro

Student Number: 0107556X

A Research Report Submitted to the School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Master of Science in Medicine in Tropical Diseases (Epidemiology and Biostatistics Option).

Johannesburg, January 2001

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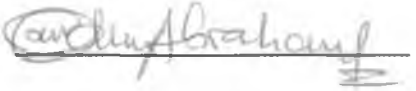
DECLARATION

I, Abraham Rexford Oduro declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine in Tropical Diseases (Epidemiology and Biostatistics Option), in the School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other university.

SIGNED:  AT JOHANNESBURG

DATE: 15th January 2002

DEDICATION

This research report is dedicated to my beloved parents, Madam Ama Boadiwaah and Opanin Kofi Nkwantabisah through whose toil my basic qualification was made possible.

ABSTRACT:

Background: increasing spread of drug resistance among *Plasmodium falciparum* poses a serious threat to malaria treatment. The situation is complicated not only because new drugs are expensive and slow in development but also because they must be effective, preferably have a novel method of action, with an acceptable level of adverse effects, and be deployed in such a way as to prolong their use.

Study Objectives: The study objective was to review and quantify the existing evidence that atovaquone plus proguanil for the treatment of *Plasmodium falciparum* malaria is a novel, safe, effective combination.

Methods: Literature search, screening, selection, extraction and met-analysis were employed in reaching the study objectives. The main outcome measures were the day 28 cure rates, mean fever and parasite clearance times. Also compliance and the incidence of adverse effects were assessed.

Results: using data from 5 pivotal randomised clinical trials; atovaquone proguanil was effective with a cure rate of 98.2% (373/380: 95%CI 96.2, 99.3). The mean fever and parasite clearance times were 32.8 hours (95% CI 30.1, 35.4) and 64.1 hours (95% CI 62.3, 65.9) respectively. Response rate was 91.3% (380/416) with 3.4% (14/416) withdrawals and 5.3% (22/414) loss to follow-up. The main adverse effects were abdominal pain (16.9%), vomiting (13.6%) and headache (12.1%)

Conclusion: Atovaquone-proguanil is effective and safe but currently expensive and potentially susceptible to resistance if deployed alone. It must therefore be reserved as a treatment alternative for patients who fail the existing malaria treatment, and must be protected by combining it with other antimalarials.

ACKNOWLEDGEMENTS

This report is based on my previous experience and the knowledge acquired in the last twelve months of my studentship at the University of Witwatersrand, School of Public Health. Johannesburg. Since joining the school, I have learned much from my friends, colleagues and most especially from my dedicated teachers to whom I am ever grateful and indebted. God bless them all.

My deepest thanks are expressed to my supervisors Drs. Robin Elisabeth Heubner and Brendan Girdler-Brown who also happened to be my Course co-ordinator. To them I say I owe you tremendous debts of gratitude.

I, wish also to express my sincere and special thanks to my colleagues and friends Dr. Victor Onwukwe, Dr. Simeon Odugwu and Mr. Abdulla Bakari Mkopi for their invaluable support, assistance and comfort to me during the painful and trying moments of my stay in Parktown Village II Residence. Words alone cannot express my gratitude to them.

In furthering my education I have been fortunate to have received a full bursary from the UNDP / WORLDBANK / WHO Special Programme for Research and Training in Tropical Diseases (TDR). I thank them for their continued assistance in building capacity in tropical diseases control and research.

FINALLY AND MOST IMPORTANTLY, I HAVE BEEN BLESSED WITH A GOD WHOSE GRACE AND LOVE TO MANKIND AND ME IS IMMEASURABLE. TO MY OMNIPOTENT GOD I SAY I WILL FOREVER BOW.

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1.0 INTRODUCTION:

1.1 BACKGROUND:

Malaria remains a major global public health problem. It is currently endemic in over one hundred countries, and an estimated 2400 million people constituting about forty percent of the world's population live in areas at risk [1,2]

The World Health Organisation (WHO) estimates that between 300-500 million clinical cases occur annually, resulting in over one million malaria deaths, each year: there are about 3,000 malaria deaths per day, world-wide, mostly in young children Malaria also imposes a huge socio-economic burden on families and governments in endemic areas through lost productivity and high health care costs [1,2].

The current resurgence of the disease is due in part to the rapid urbanisation and population growth, increased migration, dwindling financial and political support, and civil strife. In addition to deteriorating public health services and poor environmental practices, also mosquito vectors are developing resistance to commonly used insecticides, and the *Plasmodium falciparum* parasite is developing resistance to existing antimalarial drugs.

The increasing spread of drug-resistant *Plasmodium falciparum,* the predominant parasite of the four plasmodia species *(P. falciparum, P. vivax, P. ovale and P. malariae)* that cause malaria, is one of the greatest challenges currently facing most malaria control programmes worldwide [2,3,4].

Moreover, the disease is currently extending its incidence, as the epidemic is no longer a rural and focal disease. Malaria is now a serious health problem in many tropical urban centres where the vector, the anopheline mosquito, can find standing water to breed [5].

Further the increase in endemic malaria has been accompanied by a significant increase in global travel, resulting in thousands of cases being transported to non-endemic areas, a few giving rise to transmission by indigenous mosquitoes [6,7].

With currently no effective vaccine in sight and the malaria situation remaining difficult to manage, many millions of people are at risk. The major impact of the global malaria situation is, however, being felt in tropical and subtropical Asia, parts of south and Central America, and in Africa south of the Sahara, requiring special attention [2,6].

1.2 THE AFRICA MALARIA SITUATION:

Most of the world's malaria cases occur in Africa. Of the 100 countries in the world that are considered malarious about half are in Africa south of the Sahara. Also over 90% of the worlds' malaria deaths and about 80% of the annual worlds' clinical cases occur in Africa [1, 2].

Economically, it is a significant factor in lost productivity and opportunities for investment as it continues to be an impediment to tourism and foreign direct investment in Africa.

The estimated costs of malaria, in terms of strains on the health systems and economic activity lost are enormous. Malaria is estimated to account for about ten percent of the continents' disease burden, about 40 percent of all outpatient visits and 3 out of 10 hospital admissions in most endemic areas.

In Africa, where malaria reaches a peak at harvest time and hits young adults especially hard, a single bout of the disease costs an estimated equivalent of 10 working days. It is also estimated to costs rural households on average five to ten percent of the household income on treatment and prevention.

Malaria costs sub-Saharan African economies over two billion United States dollars annually, including costs for control and lost workdays: this is estimated to be 1-5% of gross domestic product and slow economic growth by about 1.3 percent per year in endemic countries [2,8,9,10].

In Africa, people most at risk are the poor, the marginalized, pregnant women and young children. African children under five years of age are chronic victims of malaria; often suffering an average of up to six attacks a year.

Malaria is a major cause of impaired childhood development and absenteeism in schools across Africa south of the Sahara. The vast majority of cases and deaths occurring among young children in Africa are in remote areas with poor access to health services [2,6,10].

The African situation is largely fuelled by the existence of suitable disease vectors and environments that are highly favourable for transmission, including poor socio-economic development and the absence of adequate health systems to deliver control measures. In addition to these, are the poor public awareness, numerous conflicts and civil wars, population movements, rapid urbanisation and widespread *P. falciparum* chloroquine resistance [2,6,10].

The malaria situation in Africa has been acute-on-chronic and is believed to be one of the major impediments to the continent's human, material and socio-economic development.

1.3 MALARIA CONTROL AND PREVENTION:

Current efforts, strategies and approaches at malaria control and prevention have focussed on providing early diagnosis and prompt treatment, planning and implementing selective and sustainable preventive measures like personal protection and vector control, and to detect early, contain and prevent epidemics. In addition to the strengthening of local capacities in basic and applied research to permit and promote the regular assessments of the malaria situation, in particular the ecological, social and economic determinants of the disease, are receiving attention and emphasis [2,11,12].

Despite these efforts, malaria has re-emerged and the control situation is worsening due to a variety of economic, environmental and clinical factors, notably the spread of drug resistance.

Malaria parasite resistance to the existing drugs is posing an increasing threat to successful malaria case management that plays a crucial role in control programmes. The once effective drug chloroquine is no longer useful in many areas. Also the effectiveness of amodiaqiune is declining due to drug resistance, as is that of sulfadoxime-pyrimethamine, especially in those countries that have adopted it as their first line drug. Many of the newer, efficacious, drugs tend to be unaffordable in poorer countries. The situation is very serious and is having a striking impact on childhood morbidity and mortality in most endemic areas. [13,14,15]

Urgent and novel strategies are needed to contain the malaria drug resistance situation in order to forestall the ongoing disaster, and though no satisfactory strategy has yet emerged. Combination treatment has been proposed in response to the situation.

This study therefore reviewed the rationale for advocating the use of combination therapy for malaria treatment and the existing evidence that atovaquone plus proguanil is a novel, effective and safe combination.

The ultimate objective however, was to pool and quantify the available data concerning the clinical and parasitological efficacy and safety of the atovaquone plus proguanil combination for the treatment of acute *Plasmodium falciparum* malaria.

1.4 RATIONALE FOR MALARIA COMBINATION THERAPY:

The useful life span of any anti-microbial agent depends on how it is developed, deployed and used. These principles must therefore underline any measure to combat the spread of drug resistance. Measures currently proposed to deal with the malaria parasites resistance situation are; to ensure correct and effective treatment of acute malaria cases, to promote the rational use of anti-malarials, and to develop new antimalarials and the use of combination therapy.

Combination therapy is given priority for various reasons.

Firstly, the slowness and high cost of new drug development, is a problem, especially since malaria is predominantly a disease of the poor: hence new drug development is economically unattractive. In addition, there is limited capacity for research and drug development in endemic areas. Thirdly, it is extremely difficult to enforce rational and good drug use practices in endemic areas due to high illiteracy rates. Moreover in most of these areas there are often limited formal health facilities for malaria case management, access may be poor, and patients

have to rely on informal suppliers: quality, dosages and efficacy of such drugs may be poor and may aggravate the spread of drug resistance.

What therefore appears feasible now is to deploy anti-malarials in such a way as to protect them and to prolong their useful life span, although no satisfactory strategy has yet been established. Combination malaria treatment defined as the use of two or more antimalarials with independent sites of action is currently seen as the best option [16,17,19]. Therefore prolonging drugs lifespan by combining them is a fundamental disease control strategy, at least in important diseases like malaria.

Drug resistance develops when spontaneously occurring parasite mutants with reduced susceptibility are selected and then transmitted. Drugs for which a single point mutation confers a marked reduction in susceptibility are particularly vulnerable. Low clearance and shallow concentration effect relationships increase the chance of selecting mutant strains [16,17].

Therefore combining anti-malarial drugs that do not share the same resistance mechanisms would reduce the chance of selection. This is because the chance of a resistant mutant surviving is the product of the per parasite mutation rates for the individual drugs, multiplied by the number of parasites in an infection that are exposed to the drugs [16,17,19].

For example, if 1 in 10^6 parasites is resistant to drug A, and 1 in 10^8 is resistant to drug B, then 1 in 10^{14} will be simultaneously resistant to the drug A-drug B combination, so long as the two drugs have independent sites of action. Since most patients with malaria have between 10^8 and 10^{12} asexual parasites in the body, a doubly resistant parasite is predicted to be around 10^{20}. This high number

of mutations needed to express total resistance will delay the development of resistance [16,17,19].

Combination treatment will also offer complete and rapid eradication of parasite load in symptomatic patients and thus reduces the chance of survival of resistant strains[16, 17,19]. It will also improve cure rates and hence lower post-treatment transmission of the plasmodia gametocytes [18].

Moreover, this basic principle underlines the current treatment recommendations for tuberculosis, HIV/AIDS and many cancers. The use of these combinations has been effective, safe and accepted worldwide.

Again recent evidence from southeastern Asia suggests that malaria drug resistance has slowed down when combination therapy of the artemisinins and mefloquine was used to treat the multi-drug resistant *Plasmodium falciparum* malaria in that area [20,21].

What remains to be determined however is the optimum combination. Current strategies have been focussed mainly on the use of the artemisinin derivatives with either sulfadoxime-pyrimethamine or mefloquine. Nonetheless, sulfadoxime-pyrithamine and mefloquine are long acting drugs with long terminal half-lives, which make them a risk for resistance selection and renders their combinations short gap measures, although the search for optimum combinations and alternatives continues [19,20].

Atovaquone-proguanil is one combination with novel structures that has potential for new combination therapy. This combination is very effective, safe, short acting, synergistic and has a novel mechanism of action. In addition, it has been found in small studies in Africa and elsewhere to be extremely safe and efficacious.

However, the individual evaluative studies have often been small in sample size and thus lack the necessary statistical power for adequate inference.

This review and meta-analysis therefore systematically reviews and quantifies the novelty, efficacy, safety or otherwise of the atovaquone plus proguanil combination for the treatment of *Plasmodium falciparum* malaria.

1.5 REVIEW OF ATOVAQUONE, PROGUANIL AND THEIR COMBINATION:

The emergence and spread of multi-drug resistant *Plasmodium falciparum* in most parts of the world has put at the forefront a compelling need to develop new antimalarials with novel structures that will be useful for limiting the morbidity and mortality of malaria infection. However to accomplish this, it is critical that compliance to the final therapy dosing must be high, and treatment regimens must be well tolerated and of relatively short duration. There is also a need to identify and include compounds that provide synergistic activity against the resistant strains with convenient regimes and acceptable safety profile. Atovaquone-proguanil hydrochloride combination for malaria treatment appears to satisfy most of these conditions as detailed below.

ATOVAQUONE: This is one of a series of synthetic hydroxynaphthoquinones found to have potent activity against *Pneumocystis carinii, Toxoplasma gondii* and *Plasmodium falciparum.* It is active against the erythrocytic stages of plasmodium development and the early gametocyte stage. It has a novel mechanism of action by depolarising parasitic mitochondria and inhibiting the electron transport system at the level of cytochrome bc_1 complex. In malaria parasites, there is obligatory coupling of pyrimidine biosynthesis and electron transport *via* ubiquinone/ubiquinol

[21,23]. The selective toxicity of atovaquone towards *Plasmodium falciparum* is achieved by virtue of the different sensitivities of mammalian and plasmodial electron transport systems to hydroxynaphthoquinones, and also by the fact that plasmodium species are dependent on *de novo* pyrimidine biosynthesis, while mammalian cells are able to salvage and recycle pyrimidines. The inability of malaria parasites to salvage preformed pyrimidines, results in atovaquone blocking nucleic acid synthesis, and thus replication, in plasmodial cells [21-23].

Pharmacokinetically however, atovaquone is characterised by relatively poor bioavailability. The oral bio-availabity is approximately 23%, which can be increased by 3 to 6 fold when taken together with a fatty meal. It is further characterised by low steady-state plasma concentration and high plasma protein binding. Excretion is almost exclusively through faeces with no significant hepatic metabolism or renal excretion. The half-life ranges from 50 to 70 hours. In a pharmacokinetics studies in malaria patients,the $C_{max,}$ which is an important determinant of therapeutic outcome was 2.8hours and mean half-life 31.8hours[26-29].

The drug is currently used as monotherapy for the treatment of *Pneumocystis carinii* pneumonia.

It should be noted that no other antimalarial has atovaquones' mechanism of action and this may explain the low incidence of its resistance worldwide and makes it a suitable candidate for drug combinations [25,26,27].

PROGUANIL: It is a biguanide derivative, first synthesised and assessed initially for the treatment of acute *Plasmodium vivax* malaria. Since then it has been used in prophylaxis, initially as a single agent and lately in combination with chloroquine, and has proved to be extremely well tolerated alone and in combination. It is metabolised *in vivo* principally by P- 450 iso-enzyme 2C19 to cycloguanil, an active metabolite, which is a potent inhibitor of dihydrofolic acid reductase.

Proguanil and cycloguanil are active against erythrocytic and extraerythrocytic stages of plasmodium development and are described as slow blood schizonticidal agents. Proguanil is well absorbed, achieving C_{max} within 2 to 5 hours.

It is metabolised in the liver to the dihydrofolate reductase inhibitor, cycloguanil. The transformation to cycloguanil is very rapid. The cycloguanil C_{max} occurs one hour after the C_{max} of Proguanil. The metabolism of proguanil is mediated by CYP3A4 and CYP2C19 isoenzymes. The later is a potential site for drug interaction.

There is considerable genetic polymorphysim of this CYP enzyme, with up to 20% 'poor metabolisers' in Asian and African populations. Poor metabolisers have very low or undetectable plasma concentrations of cycloguanil. The polymorphism may be a cause of failure in prophylaxis in poor metabolisers.

The half-life of proguanil is 12 to 20 hours in patients with malaria and healthy volunteers but longer in poor metabolisers. The half-life of cycloguanil is approximately 12 hours. Proguanil is safe and synergistic toxicities have not observed when proguanil was combined with other antimalarials hence its current combination with atovaquone [26-31].

ATOVAQUONE PLUS PROGUANIL COMBINATION: Atovaquone and Proguanil hydrochloride has been developed as a fixed drug combination of the two antimalarial agents by Glaxo Wellcome (Malorone™) and has been approved in the United States by federal drug administration for oral prophylaxis and the treatment of malaria due to *Plasmodium falciparum,* including resistant strains [30,31,32].

The rationale for combining these 2 types of folate synthesis inhibitors is however due to their synergistic activity and the fact that different genes express their resistance, which reduces the chance of selection of resistant strains.

Though resistance to some folate inhibitors has developed in most endemic areas of the world, the degree of this resistance differs markedly. Atovaquone and Proguanil work to interfere with 2 different pathways involved in the biosynthesis of pyrimidines in the malarial parasites. In the plasmodia, atovaquone selectively inhibits mitochodrial electron transport, reduces pyrimidine biosynthesis, collapsing mitochodrial membrane potential thereby preventing parasite replication.

Cycloguanil, the active metabolite of proguanil, inhibits dihydrofolate reductase, leading to depletion of pyrimidine nucleotide pools and disruption in nucleic acid synthesis and cell replication [26, 29-32].

In addition atovaquone and proguanil act synergistically against the erythrocytic stages of the parasite. Though the mechanism of this synergistic activity has not been precisely determined, *in vitro* studies suggest that the proguanil-specific synergistic effect with atovaquone is not so much dependent on its active metabolite, cycloguanil, but rather on its own specific biguanide effect. It is explained that proguanil, rather than cycloguanil, lowers the concentration of

atovaquone needed to collapse mitochondrial membrane potential in malarial parasites.

This is an advantage in that the combination will still be effective in cycloguanil resistance and also genetic polymorphism for the CYP2CIP isoenzyme or its inhibition by other drugs would not affect their efficacy [22,26,33].

Pharmacokinetics: The combined administration of atovaquone and proguanil does not change the pharmacokinetics of either drug. Atovaquone absorption from the gastrointestinal tract remains poor but increases with a fatty meal. The drug is still excreted mostly unchanged in faeces with an elimination half-life of 2 to 3 days, but low plasma levels may persist for several weeks. While proguanil is rapidly absorbed from the gastrointestinal tract, reaching peak plasma levels in 2 to 4 hours, it is concentrated in erythrocytes, metabolised in the liver by CYP2C19 and excreted in urine with half-life of 12 to 21 hours [26,29,30,34].

Uses: Atovaquone and proguanil hydrochloride is used in adults and children weighing 11 kilogram or more for chemoprophylaxis of malaria caused by *Plasmodium falciparum* and for the treatment of acute, uncomplicated malaria caused by *Plasmodium falciparum*. It is however noted that more evidence is needed on either drugs' activity against *Plasmodium vivax* hypnozoites. Neither atovaquone nor proguanil is active against hypnozoites, so the combination can neither cure nor prevent delayed primary attacks or relapse of *P. ovale* or *P. vivax* malaria. However it is the drug of choice for presumptive self-treatment of travellers to areas where resistance has been reported.

The recommended adult dosage for the treatment of uncomplicated attacks of malaria caused by *Plasmodium falciparum* is one gram of atovaquone and four

hundred milligrams of proguanil hydrochloride once daily for 3 consecutive days. Alternatively, adults may receive 500 mg of atovaquone and 200 mg of proguanil hydrochloride every 12 hours.

Children weighing 11-20 kg may receive 250 mg of atovaquone and 100 mg of proguanil hydrochloride once daily for three consecutive days: those weighing 21 - 30 kg receive 500 mg atovaquone and 200 mg proguanil, children 31-40 kg receive 750 mg atovaquone and 300 mg of proguanil hydrochloride. Children weighing more than 40 kg may receive the usual adult dosage. Alternatively, children may receive half the recommended total daily dosage every 12 hours for three days [30,31].

Adverse effects: Atovaquone plus proguanil is well tolerated; abdominal pain, nausea, vomiting, diarrhoea, headache and rash have occurred and are common with the higher doses used for treatment.

Elevations of liver enzymes have occurred but vary substantially in clinical studies of malaria therapy. In most patients, the enzyme levels returned to normal during follow-up. One treatment-related death and one case of anaphylaxis have been reported to date. [30,31,32]

Pregnancy: Proguanil alone is studied and considered safe for use in pregnancy but the safety of atovaquone and the combination is as yet unknown [30,31].

Drug Interactions: Drugs affecting the hepatic microsomal enzymes that are inhibitors of substances of the cytochrome P-450 (CYP) isoenzyme are potential pharmacokinetic interactors because they can alter proguanil metabolism. However to date no known proguanil drug interactions have been reported. Co-administration of metoclopramide with atovaquone decreases atovaquone bioavailability. It should be used concomitantly only if other antiemetics are not available.

Rifampicin has pharmacokinetic interactions with atovaquone and reduces plasma concentration of atovaquone by about 40% to 50%. Concomitant administration is therefore not recommended. Also tetracyclines have pharmacokinetic interactions with atovaquone and decrease plasma atovaquone concentration. If concomitantly used then parasitaemia should be closely monitored [26,30,31,32]

Clinical Trials: Many pivotal clinical studies have compared atovaquone plus proguanil to other drugs for the treatment of *Plasmodium falciparum* malaria. In three of such studies atovaquone/proguanil treatment led to higher cure rates than mefloquine (100% versus 86%)[35], amodiaquine (98% versus 81%)[36] and another chloroquine with pyrimethamine/sulfadoxine (100% versus 88%)[37]. In yet another three studies, atovaquone/proguanil was as effective as quinine plus tetracycline (98% versus 100%)[38], pyrimethamine/sufadoxine (100% versus 98.8%)[39] or halofantrine (93.8% versus 90.4%) [40].

Also the efficacy of atovaquone/proguanil has been evaluated for chemoprophylaxis in other studies. In two randomised double-blind trials involving semi-immune adults living in endemic areas. It was found that the drug is able to prevent malaria in 98% to 100% of those taking atovaquone/proguanil compared to 48% to 63% of patients treated with placebo [41,42]. A similar study in semi-immune children found atovaquone/proguanil 100% effective compared to 82% with placebo[43]. In addition, randomised trials in non-immune travellers have found the combination as effective as mefloquine as well as chloroquine plus proguanil [44] However, in such randomised studies without placebo the degree of exposure to malaria infection becomes unclear and such results may not be generalisable.

From the above and in all of its reported clinical studies and development, the combination has had excellent safety profiles. Most of the adverse experiences that have occurred so far have been those associated with the high doses used and they are always typical of malaria signs and symptoms and resolved within follow-up periods. These excellent clinical results and pharmacokinetic qualities of atovaquone and proguanil combination have demonstrated that the combination is equally and sometimes more effective and safe than the existing antimalarials.

However in almost all of these studies, the sample sizes have been small; therefore making their estimates and confidence intervals very wide and thus lacking precision and power.

There was therefore a need for a systematic review and re-synthesis of the available evidence that atovaquone plus proguanil is a novel combination for malaria treatment to produce more confident and precise estimates.

This study therefore qualitatively and quantitatively reviewed, and pooled the existing data on the efficacy and safety of the atovaquone plus proguanil combination for the treatment of *Plasmodium falciparuom* malaria using the World Health Organisation day 28 *in vivo* efficacy testing guidelines. The main objectives were to determine more precise estimates of the clinical and parasitological cure rates, the mean fever and parasite clearance times, the incidence and frequency of adverse effects and to assess the compliance to treatment and losses to follow-up.

2.0 METHODS AND MATERIALS:

The methods employed in the study involved systematic literature review and meta-analysis of selected pre-existing published articles. These methods as outlined below and in the subsequent paragraphs in this research report were based on the modified versions of the methods described for use in epidemiology by authors in this field [46-52]

2.1 STUDY DESIGN, SITES AND POPULATION:

DESIGN: The design involved an in-depth qualitative and quantitative review using structured guidelines and meta-analysis of results of the pre-existing independent published studies on atovaquone-proguanil combination and a comparator drug for the treatment of acute *Plasmodium falciparum* malaria. The pre-existing studies were such that all adult patients were randomly assigned to receive either atovaquone thousand milligrams per day total dose co-administered with proguanil four hundred milligram per day total dose for three days or the comparator drug for its standard regimen and duration. Children were dosed according to their body weight, with a target dose of 20 mg/kg atovaquone and 8-mg/kg proguanil. They must have all been followed up for 28 days. Primary efficacy was determined by day 28 cure rate using the World Health Organisation *in vivo* testing guidelines. Secondary efficacies were based on parasite and fever clearance times. Also incidence and frequencies of adverse experiences were determined using those that were passively reported by participants and also from clinically significant laboratory results from routinely collected study specimens.

SITES AND SETTING: Studies were selected from all malaria endemic sites across the world. However only five studies from five multiple sites qualified for inclusion. Three were in Africa (Gabon, Zambia and Kenya) [36,39,40], one in Southeast Asia (Thailand) [35] and the other in South America (Brazil) [38].

They consisted of five comparative clinical efficacy studies of acute *Plasmodium falciparum* malaria, using treatment regimen of atovaquone plus proguanil combination and different comparator drugs. All the selected studies or trials were open-label, randomised clinical trials reported during the last ten years.

POPULATION: The study population consisted of the participants in the selected studies. These consisted of female and male patients, children and adults between the ages of 3 and 65 years. They were all diagnosed as having acute *Plasmodium falciparum* malaria that was confirmed by both clinical and parasitological evidence. Only the five pivotal clinical trials / studies undertaken from 1993 to 1996 and which fully met all the selection criteria were included in the final meta-analysis.

2.2 DATA SEARCH, SELECTION AND EXTRACTION

DATA SEARCH STRATEGY: The search strategy employed was to first conduct a formal computerised literature (Medline®) search for published articles using the following key words: malaria, atovaquone, proguanil, atovaquone-proguanil, malarone and combination treatment. These key references were then examined for additional relevant references, and the process continued until no further new published articles were obtained. To minimise the shortcomings of this method, efforts were made to distinguish between independent studies and those repeated several times in the literature. Also literature from other databases(such as Pub

med and cochrane library), local journals, unpublished results and those from negative association or results that were not indexed were vigorously sought for by electronic mail communication with experts in the field and by ordering some through the school library to avoid publication bias. Only articles in English were used, as the literature search did not find any article in other languages.

STUDY SELECTION CRITERIA: Studies were included if they met all of the following criteria:

(a). Randomised controlled trial

(b). Study was on acute *Plasmodium falciparum* malaria.

(c). Atovaquone-proguanil had been compared to another antimalarial drug.

(d). Malaria case diagnosis included parasitological confirmation.

(e). The results contained information on participants' demographic characteristics.

(f). The study had measured the day 28 safety and efficacy of atovaquone-proguanil in participants as a primary outcome measure.

(g). The study had information on parasite and fever clearance times.

(h). There was information on adverse effects or experience.

(i). There was information on the treatment schedules and follow-ups.

(j). There was the existence of full published or unpublished report.

DATA EXTRACTION: For each of the selected articles the following details were extracted and tabulated; the site of the study, the period of the study, the study population, the participants' basic demographic characteristics (age and sex), the sample size and the response rate, as well as the treatment doses and schedules, the comparative drugs, the follow-up period, outcome variables and efficacy

statistics. In addition, information on loss to follow-up and clinical and laboratory adverse effects were extracted.

Two independent experienced persons did the extraction using a pre-designed table and guidelines under the facilitation of the author and the direction of his research report supervisors.

SAMPLING. The final five selected articles for this research report were all pooled and included as part of the overall sample size estimation.

For a study with dichotomous outcome (cured or not cured) and based on the overall cure rates at day 28 as a primary efficacy outcome. The sample size estimation was determined as follows. Previous estimates of cure rates for atovaquone-proguanil combination were over ninety percent in most cases. Thus assuming 85% cure rate and the probability of 95% that the estimated parameter will be within 5% of the true value, the minimum number of participants or cases required from the pooled articles was 196. This was calculated using Epi-Info software. However, assuming 20% loss to follow-up, and for the purposes of non intention-to-treat analysis the total minimum pooled cases required were 236.

2.3 DATA MANAGEMENT:

Only results from the five selected studies were included in the final data management and re-synthesis. All the essential information needed was initially used in the literature review and then extracted, tabulated and pooled. They were then re-synthesised into one measurement. Qualitative and quantitative methods were used in all stages of data management. For the qualitative assessment the independent reviewers applied the checklist and the scoring to finally select the five articles. However for the quantitative analysis, all the reported estimates were

extracted and based on the studies objectives the essential data was pooled and later resynthesied.

QUALITATIVE ASSESSMENT: Reports of all the retrieved literature were reviewed and qualitatively assessed using a review checklist system as detailed figure 1.

They were later sent to independent reviewers for final review and scoring according to their quality. Below is the checklist and scoring system used.

The Qualitative Checklist and Scoring System used:

(a). Clearly stated objectives; yes or no (1,0)

(b). Study design; randomised, placebo-controlled, blinding. (1 each)

(c). Defined accessible population; yes or no (1,0)

(d). Subject demographic characteristics stated; yes or no (1,0)

(e). Subject selection; probabilistic, consecutive, others (3, 2,1 respectively)

(f). Sample size adequacy; yes or no (1, 0)

(g). Response rate; ≥ 75 % or less (1,0)

(h). Malaria case diagnosis; appropriate or inappropriate (1,0)

(i). Adequate treatment procedures; yes or no (1,0)

(j). Sufficient follow-up: yes or no (1,0)

(k). Outcome variables; appropriate or inappropriate (1,0)

(l). Unethical issues; yes or no (1,0)

(m). Systematic error; none, minimal, moderate and severe (4,3,2,0)

(n). Appropriate statistical test; yes or no (1,0)

(o). Adequate data analysis; yes or no (1,0)

(p). Adequate level of statistical precision; yes or no (1,0)

(q). Consistent reporting: yes or no (1,0)

QUANTITATIVE ASSESSMENT: This involved extracting all numerical values including the point and interval estimates of the selected articles. These include the period of study, number of males and females, the age range, mean age and its standard deviation, the mean weight with its standard deviation and the geometric mean and range of parasite counts. In addition treatment doses, duration and follow-up were also extracted. Also the number, which completed 28 days follow-up, the number cured, the cure rates, the fever and parasite clearance times were noted.

Further, total withdrawals, loss to follow-up and the frequency of adverse effects were recorded. The extracted data was tabulated and resynthesised. Rows were created for each quantitative variable and separate columns were allocated for each of the selected articles or studies.

STATISTICAL ANALYSIS: This involved all the data with summary measures for the separate and pooled results. Since the studies were all estimating day 28 cure rates and their sample sizes were comparable, the homogeneity assumption was made and the fixed effect model used[47] by pooling their standard deviations using the formula $SD_p = \sqrt{\{(n_1-1)SD_1^2 + (n_2-1)SD_2^2 +,..+ (n_X-1)SD_X^2\}/(n_1+n_2+,..+n_x)}$[53]. Hence weighted averages were estimated for the entire reported estimates and their confidence constructed after estimating the pooled standard deviation and error using the pooled formula [53].

The pooled average-weighted estimates and confidence intervals for the mean age, weight and parasite counts and their ranges were all determined using stata statistical software and presented.

For the treatment outcomes graphical methods of display using all the clinical outcomes were estimated for the individual and the combined results.

The raw data were also combined and reanalysed. The 95% confidence intervals for the combined results were portrayed. Overall statistical significance/ pooled measures of effects estimated were used for comparing comparator drugs.

Since the compliance of a treatment regimen mirrors its acceptability and hence final effectiveness in the field, a null hypothesis of no difference between efficacy and effectiveness were tested to see if there was a clinically significant difference between the two cure rates.

All the results were later discussed and inferences drawn. In addition, the limitations of the study were stated. References were also compiled and presented.

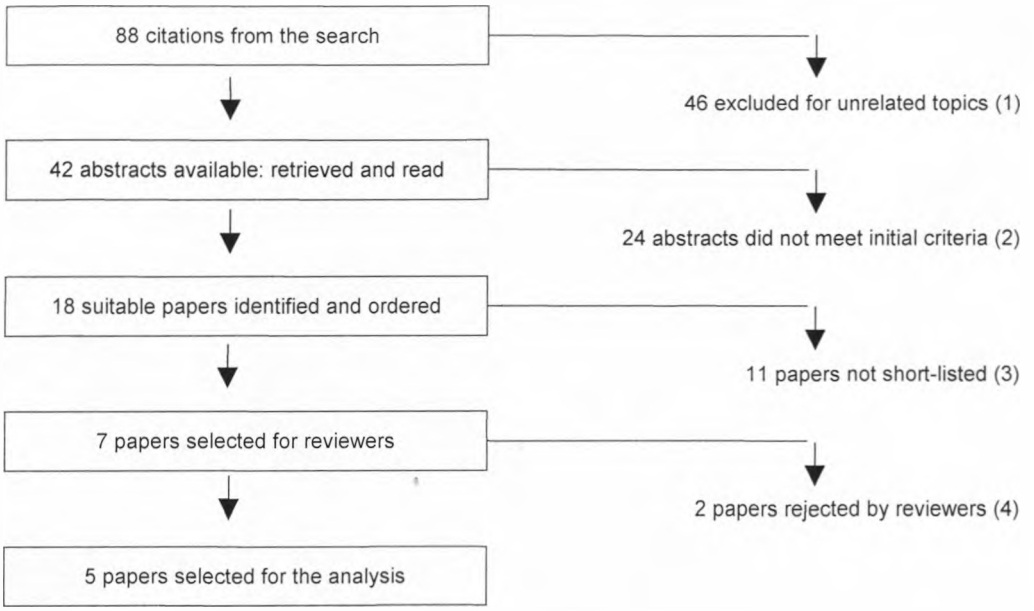
3.0 RESULTS:

The report results are grouped under three major headings: qualitative extractions, quantitative extractions and finally the study outcomes and endpoints.

3.1 THE QUALITATIVE EXTRACTIONS

Steps employed in selecting the final 5 articles for the meta-analysis

FIGURE 1: Flow diagram of steps used to select the final articles.



[1]These articles were rejected because atovaquone or proguanil was just mentioned in their write-up and not studies involving of atovaquone/proguanil.

[2] These articles had studied and reported on atovaquone/proguanil but the studies were not clinical efficacy studies

[3] Ten of these articles could not meet the initial study selection criteria as detailed in the next page and one article from Peru did not arrive.

[4] These two articles did not meet the minimum required qualitative score of 18.

The table below gives the background information of the 5 final selected articles.

TABLE I: The Study number, site and source of the final five selected articles.

#	Site	Authors, title and Source of the journal articles selected
1	**Kenya**	Anabwani G, *et al.* Combination atovaquone and proguanil versus halofantrine for treatment of acute *Plasmodium falciparum* malaria in children. *Paediatric Infectious Diseases Journal.* 1999; 18:456-61.
2	**Zambia**	Mulenga M, *et al.* Atovaquone and proguanil versus pyrimethamine/sulfadoxine for the treatment of acute *Plasmodium falciparum* in Zambia. *Clinical Therapeutics.* 1999;21(5):841-52.
3	**Gabon**	Radloff PD, *et al.* Atovaquone and proguanil for *Plasmodium falciparum* malaria. *Lancet.* 1996; 347:1511-14.
4	**Brazil**	De Alencar FEC, *et al.* Atovaquone and proguanil for the treatment of malaria in Brazil. *The Journal of Infectious Diseases.*1997; 175:1544-7.
5	**Thailand**	Looareesuwan S, *et al.* Efficacy and safety of atovaquone/proguanil compared with mefloquine for the treatment of *Plasmodium falciparum* malaria in Thailand. *American Journal of Tropical Medicine and Hygiene.* 1999; 60(4): 526-532.

Below is the independent reviewers report on the qualitative results of the 5 final articles:

TABLE II: The Final Qualitative Scores (Score range: 19 - 21).

Criteria for qualitative assessment (score)					
Study number	**1**	**2**	**3**	**4**	**5**
Clearly stated objectives; yes or no (1,0)	1	1	1	1	1
Design; randomised/ placebo-controlled/ blinding. (1 each)	2	1	2	1	2
Defined accessible population; yes or no (1,0)	1	1	1	1	1
Basic demographic characteristics stated; yes or no (1,0)	1	1	1	1	1
Subject selection; probabilistic, consecutive or other non-probabilistic (3, 2,1 respectively)	2	2	2	2	2
Sample size estimation stated; yes or no (1, 0)	0	0	1	0	0
Follow-up rate;≥ 75 % , < less (1,0)	1	1	1	1	1
Malaria case diagnosis; appropriate or inappropriate (1,0)	1	1	1	1	1
Adequate treatment procedures; yes or no (1,0)	1	1	1	1	1
Sufficient 28 day follow-ups: yes or no (1,0)	1	1	1	1	1
Outcome variables; appropriate or inappropriate (1,0)	1	1	1	1	1
Serious unethical issues; yes or no (0,1)	1	1	1	1	1
Bias; none, minimal, moderate and severe (4,3,2,0)	3	3	3	3	3
Appropriate statistical test; yes or no (1,0)	1	1	1	1	1
Adequate data analysis; yes or no (1,0)	1	1	1	1	1
Adequate level of statistical precision; yes or no (1,0)	1	1	1	1	1
Consistent reporting: yes or no (1,0)	1	1	1	1	1
Total score	**20**	**19**	**21**	**19**	**20**

3.2. THE QUANTITATIVE EXTRACTIONS

TABLE III: The Quantitative Extractions Results:

Selected Study number	1	2	3	4	5
Selected Study site	Kenya	Zambia	Gabon	Brazil	Thailand
Selected Study period	1994-1994	1993 –1994	1994 -995	1996-1996	1993- 1994
Sample size	84	82	71	88	91
# Females	45	2	37	0	20
# Males	39	80	34	88	71
Age range in years	3-12	14-49	15-65	18-65	17-65
Mean age (years) (SD)	6.4 (2.7)	25.9 (7.8)	32(17)	30.2(9.7)	27.9(10.3)
Mean weight (kg) (SD)	18.1 (5.2)	56.3 (7.2)	59(11)	---	52.9(8.5)
Parasite geometric mean /ul	29 686	14,799	5030	12,059	38,270
Parasite range	738 – 364,928	872 - 61,813	225- 100,000	(SE 1696)	570- 198,800
Total dose (mg) (A:P)	(20:8)mg/kg*	1000: 400	1000:400	1000:400	1000:400
Treatment duration/days	3	3	3	3	3
Follow-up period in days	28	28	28	28	28
# of 28-day follow-up	81	80	63	77	79
# of patients cured	76	80	62	76	79
Cure rate in %	93.8	100	87**	98.7	100
# of patients withdrawn	3	2	0	8	1
# loss to follow-up	0	0	8	3	11
Mean Fever clearance time, hours (SD)	35.9 (28.3)	30.4 (28.2)	16(22)	18.8(17.7)	58.9(36.1)
Mean Parasite clearance time, hours (SD)	64.9 (17.3)	64.0(21.7)	72(23)	56.1(14.1)	65.2(17.6)
Compactor drug	Halofantrine	Pyrimethamine - sulfadoxine	Amodiaquine	Quinine + tetracycline	Mefloquine

* Dose per kilogram body weight for children.

** Cure rate by intention-to-treat analysis

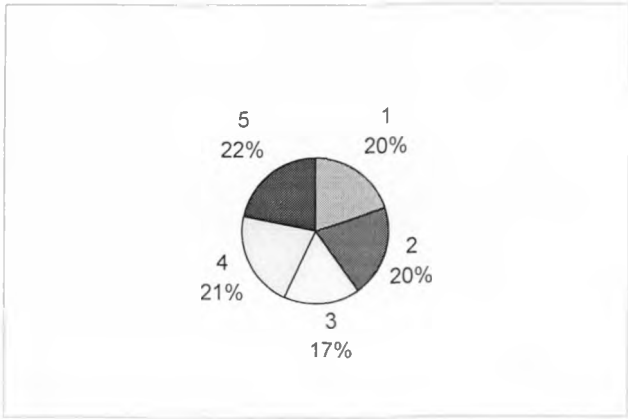
3.3 THE BACKGROUND INFORMATION:

In all, the results from five clinical trials from three continents Africa (3/5), Asia (1/5) and South America (1/5) were pooled. The percentage of participants from each of the five contributing sites were Kenya 20.1% (84/416), Zambia 19.7% (82/416), Gabon 17.1% (71/416), Brazil 21.2% (88/416) and Thailand 21.9 (91/416). All were undertaken between the periods 1990 to 2000 and had compared atovaquone plus proguanil to halofantrine, sufadoxine/pyrimethamine, amodiaquine, quinine plus tetracycline and mefloquine respectively. They were reviewed by two independent persons and satisfied all inclusion/exclusion criteria.

TABLE IV: The Site, Number and Percent Contribution to overall Sample Size.

Study Site	Study Number	Sample Size	Percent Total
KENYA	1	84	**20.2**
ZAMBIA	2	82	**19.7**
GABON	3	71	**17.1**
BRAZIL	4	88	**21.1**
THAILAND	5	91	**21.9**
Combined	(6)	**416**	**100**

FIGURE 2: Pie Chart of Percent Sample Size Per Site



POOLED PATIENTS CHARACTERISTICS: A total of 416 participants with acute uncomplicated *Plasmodium falciparum* malaria randomised to treatment with atovaquone plus proguanil only are analysed.

In all 75% (312 / 416; 95% CI 70.5, 79.1) of the total cases were males.

About 20% (84/416; 95% CI 16.4, 24.3) were between the ages of 3 to 12 years. The weighted-average estimate of the mean age was 24.4 years. (95% CI 23.4, 25.4; the pooled SD =10.3). The combined age, ranged from 3 to 65 years old.

Also the weighted average estimate of the mean weights was 46.2 kilograms (95% CI 45.4,47.0; the pooled SD = 8.1).

The minimum geometric mean of *Plasmodium falciparum* parasite count reported by the studies was 5030 per microliter and the maximum was 38,270 per microliter. However, the overall range was between 225 to 364,928 parasites per microliter.

The treatment regimens were all the same; for adults' one-gram atovaquone and 400-milligram proguanil were given together as single daily doses for three consecutive days.

However children were dosed according to their body weight, with a target dose of 20mg/kg atovaquone and 8-mg/kg proguanil.

3.4 THE PRIMARY EFFICACY ENDPOINTS:

TREATMENT OUTCOMES: The follow-up period for which treatment outcomes were measured was 28 days. Of the total 416-pooled patients in the study, 380 fully completed treatment and finished the 28 days follow-up, making the overall study response rate of about 91% (380 / 416; 95% CI 88.2, 93.7).

Fourteen of the patients, about 3.4 % (14 / 416), were withdrawn for various reasons before study completion while another 22 patients about 5.3% (22 / 416) were lost to follow-up.

Table V below gives further details of the results in terms of total sample size per study, number completed, cured and withdrawn, and the cure rates by evaluable patients and by intention to treat analysis.

TABLE V: Treatment Outcomes, Cure Rates and Confidence Intervals

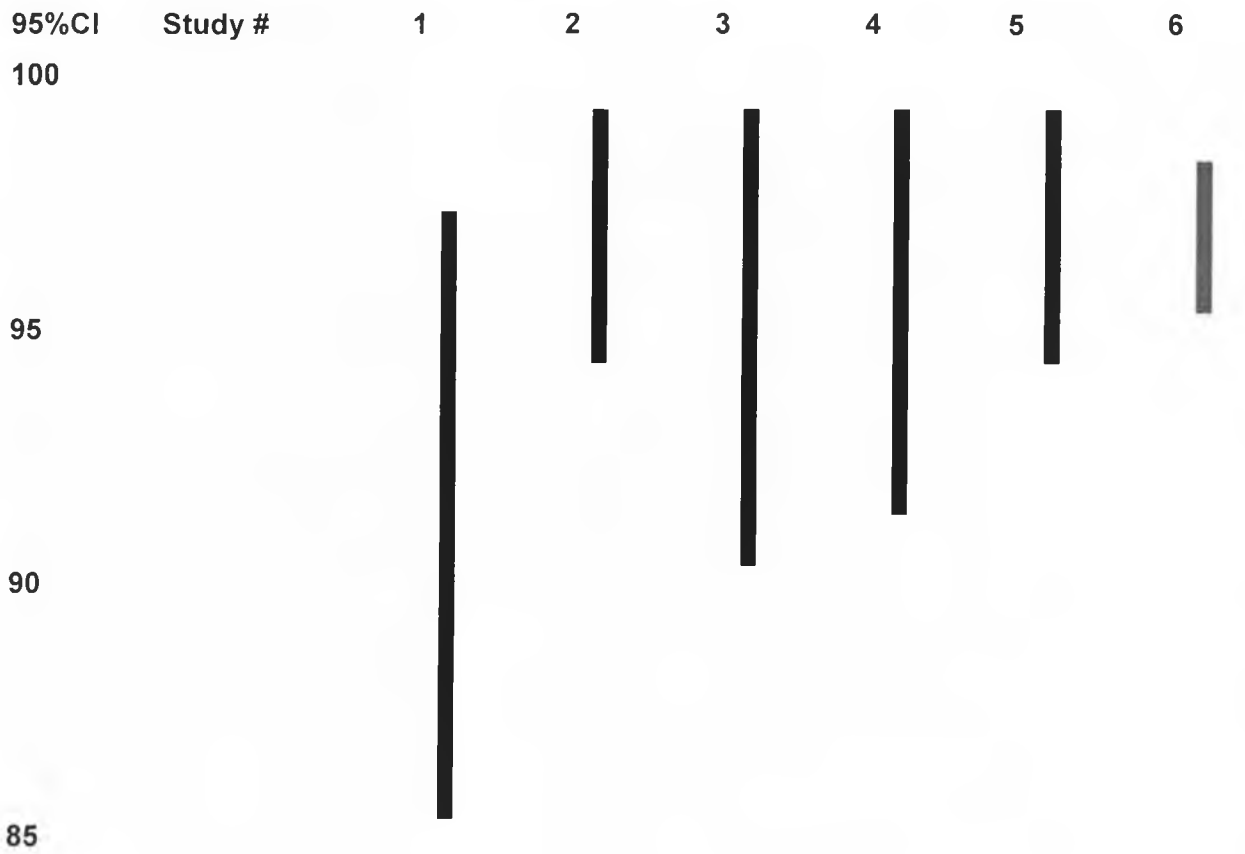
Study Site	#	Sample size	Number Completed	Number Withdrawn	Number Cured	95% CI of Cure rates	
						Evaluable subjects	Intention-to-treat
Kenya	1	84	81	3	76	93.8(86.1,98.0)	90.5(82.0,95.8)
Zambia	2	82	80	2	80	100(95.5,100)	97.6(91.4,99.7)
Gabon	3	71	63	8	62	98.4(91.4,99.9)	87.3 (77.3,94.0)
Brazil	4	88	77	11	76	98.7(92.9,99.9)	86.4(77.3,92.7)
Thailand	5	91	79	12	79	100(95.4,100)	86.8(78.1,92.9)
Combined	**6**	**416**	**380**	**36**	**373**	**98.2 (96.2, 99.3)**	**89.7(86.3,92.4)**

CURE RATES BY EVALUABLE PATIENTS (EFFICACY):

Using the WHO in *vivo* day 28 efficacy testing guideline. A total of 380-pooled patients completed the study treatment and the 28-day follow-up and were thus evaluable for efficacy analysis. Out of this number, 373 were cured of their malaria without recrudescence in the 28 days follow-up. This gives the treatment cure rate (efficacy) of atovaquone plus proguanil in the study population to be **98.2%** (373 / 380; 95% CI 96.2%, 99.3%).

TABLE V presents the precise 95% confidence intervals of the individual and combined results and figure 3 below gives the graphical presentation.

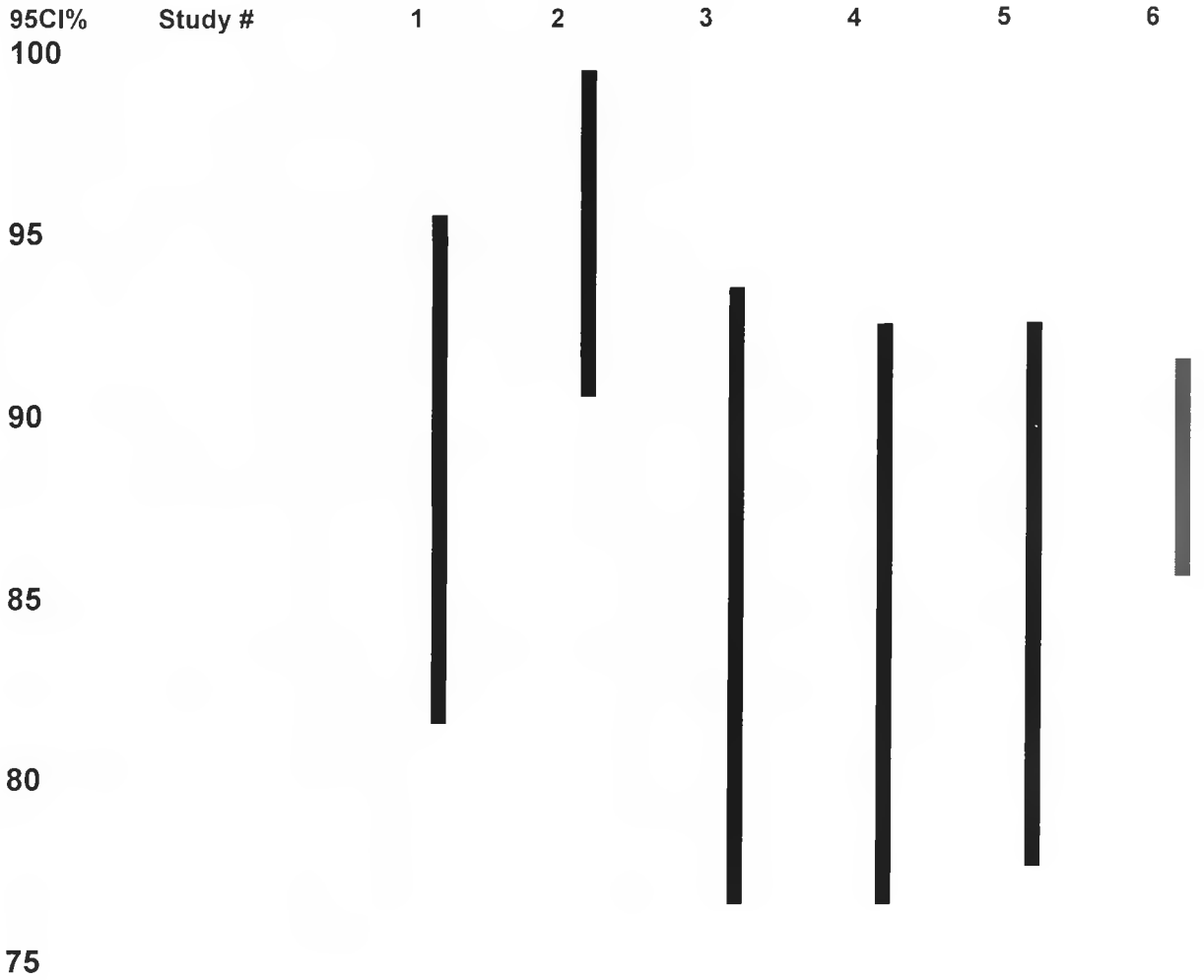
FIGURE 3: 95% CI of Cure Rates by Evaluable Patients (Efficacy)



CURE RATES BY INTENTION-TO-TREAT ANALYSIS (EFFECTIVENESS):

Intention to treat analysis to determine the effectiveness of the pooled results was also done. That is, all the randomised participants were included in the final analysis. Out of the total 416 participants initially randomised 373 were cured. This gives the estimated effectiveness of atovaquone plus proguanil in the combined study population to be **89.7%** (373/416; 95% CI 86.3% to 92.4%). TABLE V: above and Figure 4: below depict details of the 95% confidence intervals of the individual and the combined results.

FIGURE 4: 95% CI of Cure Rates by Intention-to-Treat Analysis (Effectiveness)



CURE RATES: EFFICACY VERSUS EFFECTIVENESS.

Since the compliance of a treatment regimen mirrors its acceptability and hence the final effectiveness in the field, I compare below the treatment efficacy to the effectiveness. I then tested the null hypothesis that there is no significant difference between the combined study treatment efficacy (373/380) and treatment effectiveness (373/416).

The results of a binomial exact test is significantly as follows:

95% CI for the difference = 5.3% to 11.7% ($p < 0.0001$).

The results of the efficacy and effectiveness of the individual and combined results are depicted by a bar chart; FIGURE 5 and the confidence intervals of the difference in the two cure rates are depicted on TABLE VI and FIGURE 6 in the next page:

FIGURE 5: The Comparison of the Study Efficacy and Effectiveness

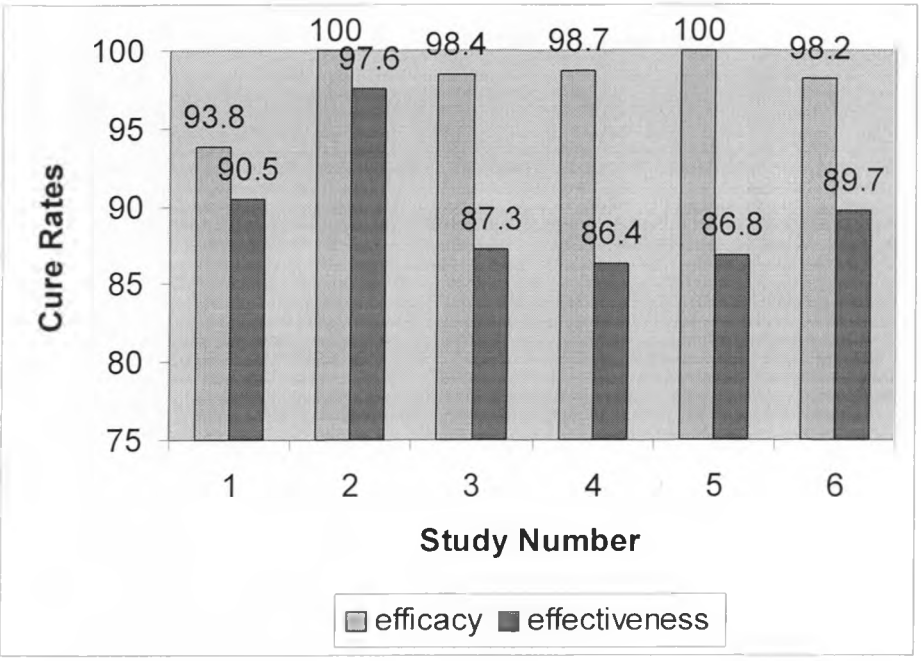
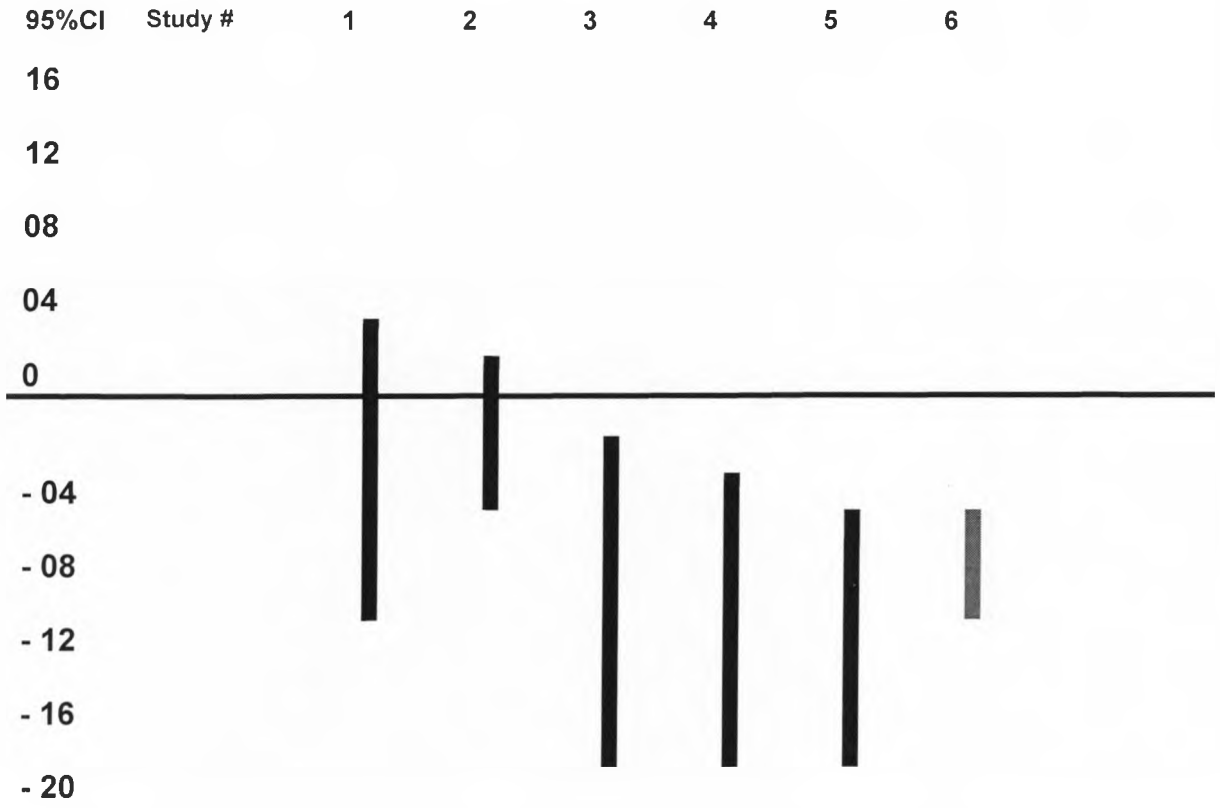


TABLE VI: Differences between the treatment Efficacy and Effectiveness

Site	#	Total size	#Completed	# Withdrawn	#Cured	95% CI of the difference
Kenya	1	84	81	3	76	-11.5, 4.83
Zambia	2	82	80	2	80	-5.7, 0.9
Gabon	3	71	63	8	62	-19.4, -2.8
Brazil	4	88	77	11	76	-19.9, -4.7
Thailand	5	91	79	12	79	-20.1, -6.2
Combined	**6**	**416**	**380**	**36**	**373**	**-11.7, -5.3**

FIGURE 6: 95%CI Differences Between Treatment Efficacy and Effectiveness



From the above; studies 1 and 2 showed no significant difference between efficacy and effectiveness. While in studies 3, 4 and 5 there were significant differences. Also, the combined (study 6) result showed a significant difference and more precise estimate with a between the two cure rates (5.3% to 11.7% $p < 0.0001$)).

3.5 THE SECONDARY EFFICACY ENDPOINTS:

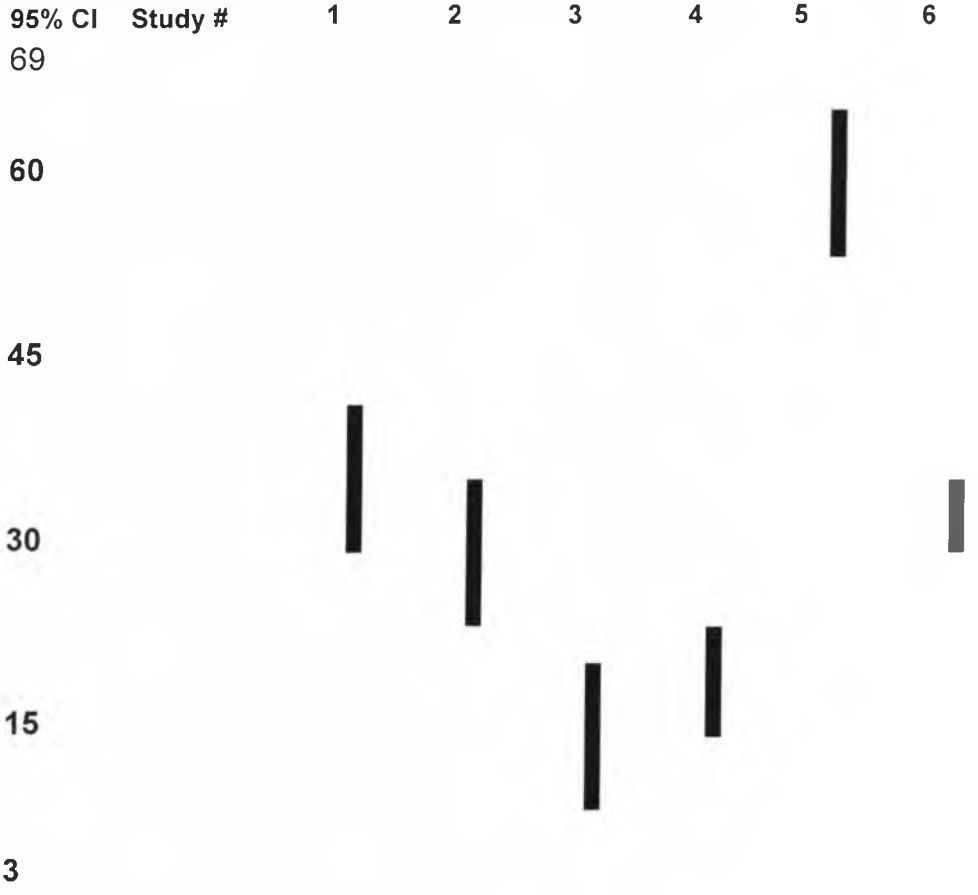
THE FEVER CLEARANCE TIMES:

The weighted-average mean fever clearance time was 32.8 hours (95% CI 30.1 to 35.4). (Pooled SD= 27.4 hours). The range of the reported mean fever clearance times was 16.0 to 58.9 hours. Below are details on TABLE VII and figure 8.

TABLE VII: Fever Clearance Times and Confidence Intervals

Study Number	Sample Size	Mean Fever Clearance Time	Standard Deviation (SD)	95% Confidence Interval
1	84	35.9	28.3	(29.7, 42.1)
2	82	30.4	28.2	(24.2, 36.5)
3	71	16.0	22.0	(10.7, 21.2)
4	88	18.8	17.7	(15.0, 22.5)
5	91	58.9	36.1	(51.3, 66.4)
6(combined)	416	32.8	27.4	(30.1, 35.4)

FIGURE 7: 95% Confidence Intervals of Fever Clearance Times



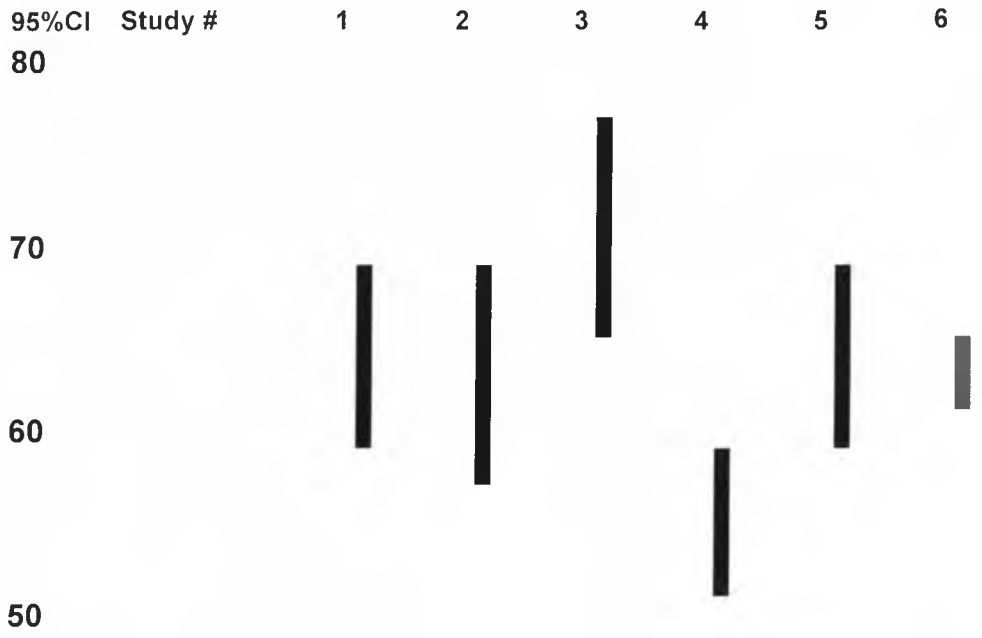
THE PARASITE CLEARANCE TIMES:

The weighted-average mean parasite clearance time was 64.1 hours (95% CI 62.3 to 65.9). The range was 56.1 to 72.0 hours. TABLE VIII and FIGURE 8 gives further details.

TABLE VIII: Parasite Clearance Times and Confidence Intervals

Study Number	Sample Size	Mean Parasite Clearance Time	Standard Deviation (SD)	95% Confidence Interval
1	84	64.9	17.3	61.1, 68.6
2	82	64.0	21.7	59.2, 68.7
3	71	72.0	23.0	66.5, 77.4
4	88	56.1	14.1	53.1, 59.0
5	91	65.2	17.6	61.5, 68.8
6(combined)	416	64.1	18.8	62.2, 65.9

FIGURE 8: 95% CI of parasite clearance times:



WITHDRAWALS:

In all 14 (3.4%) participants were withdrawn from the studies before the stipulated 28 days follow-up. The reasons for withdrawal were as follows: two participant were removed for withdrawal of parental consent, one was removed for repeatedly vomiting of the study drug, six were detected to have taken other antimalarials during the follow-up period and five were found to have developed concomitant infections.

LOSSES TO FOLLOW-UP:

In all 22 study participants, 5.3% (22/416) were lost to follow-up from the study before the end their 28 days follow-up.

3.6 SAFETY ANALYSIS:

SYMPTOMATIC SAFETY ANALYSIS:

Reported adverse experience analysis is restricted to data on participants that were reported by the selected studies. In all 397 out of the total 417 participants were reported to have complained of one or more adverse experiences. The reported adverse experiences were typical of malaria symptoms. In descending order of frequency, the 10 most common adverse experiences were abdominal pain 16.9% (67/397), vomiting 13.6% (57/397), and headache12.1% (48/397), diarrhoea and nausea were the same 9.8% (39/397). The rest were weakness 7.3% (29/397), pruritus 5.5% (22/397), anorexia and dizziness were the same 4.8% (19/397) and last but not the least coughing 2.5% (10/397). TABLE IX on the next page illustrates the detail frequencies.

TABLE IX: Reported symptomatic adverse effects; results of the selected studies

Study number	**1**	**2**	**3**	**4**	**5**	**Total**	**100%**
Sample size	84	82	63	77	91	**397**	**100.0**
Vomiting	13	10	18	4	9	**54**	**13.6**
Nausea		6	21	12		**39**	**9.8**
Diarrhoea	4	13	12	5	5	**39**	**9.8**
Pruritus	9	4	3	6		**22**	**5.5**
Headache	8	23		17		**48**	**12.1**
Abdominal Pain	8	23	14	20	2	**67**	**16.9**
Dizziness		6	3	10		**19**	**4.8**
Anorexia	3	6	5	5		**19**	**4.8**
Weakness	1	19		9		**29**	**7.3**
Coughing	10					**10**	**2.5**
Insomnia	2		5			**7**	**1.8**
Rash	3					**3**	**0.8**
Chills	2					**2**	**0.5**
Epistaxis	1					**1**	**0.3**
Myalgia		8				**8**	**2.0**
Palpitation	1	3				**4**	**1.0**
Hepatomegaly		5				**5**	**1.3**
Hypotension		6				**6**	**1.5**
Splenomegaly		3				**3**	**0.8**
Tinnitus			3	3		**6**	**1.5**
Sore throat					7	**7**	**1.8**

LABORATORY SAFETY ANALYSIS:

Only two of the selected studies (numbers 2 and 5) reported on clinically significant laboratory abnormalities. However all were the types commonly seen in malaria infections. In most of the patients the laboratory abnormalities resolved after treatment and before the end of the day 28 follow-ups. The six most significant ones in decreasing frequency were, increased eosinophil counts 27.8% (48/173), decreased neutrophil count and increased aminotransferase were the same at 10.4% (18/173). The rest were decreased albumin 9.8% (17/173), increased aspartate aminotransferase 8.7% (15/173) and decreased red blood cell count 5.9% (9/173). Refer to TABLE X below for further details

TABLE X: Clinically Significant Laboratory Abnormalities.

Laboratory test	Criteria	Study number 2 (n=82)		Study 5 (n=91)		Combined
		Developing abnormality	Abnormal at day 28	Developing abnormality	Abnormal at day 28	n=173 (%)
Haematocrit	<25%	1	0	6	0	**7 (4.1)**
Haemoglobin	<7.5g/dl	0	0	4	0	**4 (2.3)**
Red blood cell	$<3x10^{12}/L$	1	0	8	0	**9 (5.2)**
White blood cell	$<3x10^{9}/L$	2	0	3	0	**5(2.9)**
Neutrophil count	$<1x10^{9}/L$	14	4	4	0	**18(10.4)**
Eosinophil	>1000/μL	10	8	38	32	**48(27.8)**
Platelet count	$<50x10^{9}/L$			3	0	**3 (1.7)**
Creatinine	>2.0mg/dl	2	0	0	0	**2 (1.2)**
Albumin	<3.0g/dl	11	4	6	0	**17(9.8)**
Bilirubin	>2.0mg/dl			6	0	**6 (3.5)**
ALT	>100u/L	3	1	15	2	**18(10.4)**
AST	>100u/L	3	0	12	3	**15(8.7)**

4.0 DISCUSSION:

Early diagnosis and prompt treatment of malaria with effective drugs has been the cornerstone of malaria control, especially in endemic areas where for economic and other reasons, other measures of control are absent or inadequate. However the rapid development and spread of malaria parasite resistance to existing antimalarials is posing increasing threats to this control strategy. The situation is even more serious, not only because new efficacious drugs are expensive and slow in development but also because these drugs must be easy to use, have few adverse effects and be deployed in such ways as combination therapy so as to protect and prolong their useful lifespan. From the systematic review, qualitative and quantitative analysis so far, it has been shown in this research report that not only is atovaquone plus proguanil a novel combination but also it fulfils most of the criteria of combination, high efficacy, easy administration and fewer and acceptable adverse effects.

This combination is better than mefloquine plus artesunate and sulfadoxine-pyrimethamine plus artesunate combinations in terms of its synergistic activity [33] and relatively shorter terminal half-life, which is a selective pressure for drug resistance and invariably determines the useful lifespan of a given drug.

Regarding novel structures the mechanism of action of atovaquone by depolarising parasitic mitochondria and selectively inhibiting the electron transport system at the level of cytochrome bc_1 complex is unique [21]. Couple to this, is the proguanil specific synergistic effect with atovaquone, which is not so much dependent on its active metabolite, cycloguanil, but rather on its own specific biguanide effect. This is a significant advantage that will make the combination still

effective even in the context of cycloguanil resistance. Again the genetic polymorphism for the CYP2CIP isoenzyme or its inhibition by other drugs would not affect their efficacy due to this synergism [26].

On efficacy of atovaquone-proguanil, the results of this study confirm what had been previously reported. With treatment efficacy rate of 98.2% (95% CI 96.2%, 99.3%) atovaquone-proguanil has higher cure rates than current mono-therapies like mefloquine and amodiaquine, and halofantrine, which had previously been thought to be similar, although the background parasite resistance rates were not described for the various study sites. Also atovaquone-proguanil is equally efficacious to current frontline combinations like artesunate plus pyrimethamine / sulfadoxine, artesunate plus mefloquine and quinine plus tetracycline but higher than pyrimethamine /sulfadoxine plus chloroquine or amodiaqiune in the areas where the comparative studies were done [35,36,37,38,39,40].

Also to assess the combinations acceptability and compliance in the field, intention-to-treat analysis was done and then compared to cure rates due to evaluable cases only. The atovaquone-proguanil is highly effective with an intention-to-treat cure rate of 89.7% (95% CI 86.3% to 92.4%). Though the results from a binomial exact test {95% CI 5.3% to 11.7%, p-value < 0.0001} indicates that the effectiveness is significantly different from the efficacy (98.2%). Though this is a significant finding its implication on the usefulness of the drug is minimal as most of the reasons for the withdrawals and hence the difference between the combination's efficacy and effectiveness were mainly drug unrelated: two participant were removed for withdrawal of parental consent, one was removed for repeatedly vomiting of the study drug, six were detected to have taken other

antimalarials during the follow-up period and five were found to have developed concomitant infections.

the combination is still more effective than most current mono-therapies in endemic areas and is comparable to other drug combinations if intention to treat analysis were to be estimated.

The study has also been able to obtain and improve on estimates of the treatment outcomes by pooling the five studies. The study treatment efficacy of 98.2% (95% CI 96.2%, 99.3%) and effectiveness of 89.7% (95% CI 86.3% to 92.4%) is the most precise estimates compared to any individual results ever published. This is shown on tables V and VI and depicted graphically on figure 3 and 4. In all cases the confidence estimates were reduced. However I am unable to determine the precise estimates for women and children separately as not all the retrieved articles provided separate data on women and children.

On the side of the secondary efficacy parameters, the results showed that patients treated with atovaquone-proguanil have mean fever clearance times of 32.8 hours (95% CI 30.1 to 35.4). This finding is significantly shorter than fever clearance time reported in sulfadoxime-pyrimethamine, halofantrine , and mefloquine . Also the mean atovaquone-proguanil fever clearance time was comparable to that of chloroquine plus sulfadoxime-pyrimethamine [35,37,39,40].

Once again these comparative results may not be valid in areas that differ from the study areas with regard to background parasite resistance patterns. However this finding is relatively higher and converse compared to quinine plus tratracycline as

previously reported[38]. Again this finding is significantly higher than in amodiaquine alone and in combination with sulfadoxime-pyrimethamine.

Further patients treated with atovaquone proguanil have mean parasite clearance time of 64.1 hours (95% CI 62.3, 65.9) which is significantly shorter than that of amodiaquine and mefloquine[35,36]. However this finding is comparable to quinine plus tetracycline[38] initial thought to shorter and other combinations. However the combination has a higher parasite clearance time than sulfadoxime/pyrimethamine , chloroquine or their combination and halofantrine [39,40]. Parameters of fever and parasite clearance times are important, as they are suggestive of the rate of drug effect. Also delayed parasite clearance time in addition to shallow concentration effect of drugs due to long terminal half life is a strong determinant of resistant parasite selection especially in areas where malaria re-infections are common.

It is stressed that the weighted-average estimates are appropriate and are used in the statistical analysis because the study outcomes are the same justifying the homogeneity assumption and hence the invoking of the fixed effect model.

Also by pooling the various studies we have been able to improve on the precisions of the individual estimates and thus further enhance outcomes

Furthermore the difficulty in drug combination and safety were assessed. While it may appear simple to combine antimalarials for use, the procedure is often limited by drug interactions, which may alter the absorption, distribution, elimination, toxic levels or effectiveness of the individual or the combined agents. From the results of our systematic review, the combined administration of atovaquone and proguanil does not change the pharmacokinetics of either drug and also synergistic toxicities were not observed. This is so because proguanil has no

known drug interactions when combined with other antimalarials and hence its combination with atovaquone [26].

Atovaquone and proguanil were both well tolerated. Though 397 out of the total 417 participants complained of one or more adverse experience, all of them were typical of malaria symptoms. The three most common adverse experiences were abdominal pain, vomiting and headache. Others were diarrhoea, nausea, weakness, pruritus, anorexia and dizziness. The percentage of abdominal pains is 16.9 (95% CI 13.3.20.9) smaller than found in halofantrine, sulfadoxine/pyrimethamine and quinine plus tetracycline whilst higher than found in amodiaquine and mefloquine. The higher frequency of abdominal pain can however be attributed to the number of tablets patients have to swallow.

Also about 14% (95%CI 11,18) complained of vomiting which is higher than that found in mefloquine and halofantrine but similar in sufadoxine/pyrimethamine and amodiaquine and smaller in quinine plus tetracycline. Again about 12% (95%CI 9,16) reported of headache, this is similar in halofantrine and quinine plus tetracycline but smaller in mefloquine [35,36,37,38,39,40]. These and the other findings are however related to malaria treatment and do not point to any specific toxicity. Two of the selected studies also reported on significant laboratory abnormalities, the results showed that significantly 27.7% had raised eosinophils, however this is attributed to the high prevalence of helminthic infections in the areas of study.

Also about 10% had raised liver enzymes and raised neutrophil counts while about 9% had decreased albumin. All did not differ much from the comparable drugs, and all resolved except the eosinophils within the follow-up period.

In all they were not clinically symptomatic.

To determine effectiveness, the adequacy of response rate in the studies was assessed. In all about 91.3% achieved the study primary endpoint. However about 3.4% (14/416) participants were withdrawn before the stipulated follow-up. The reasons for withdrawal were as follows: two participant were removed for withdrawal of parental consent, one was removed for repeatedly vomiting of the study drug, six were detected to have taken other antimalarials during the follow-up period and five were found to have developed concomitant infections.

Also 22 study participants, 5.3% (22/416) were lost from the study before the end their 28 days follow-up. The above findings suggest that atovaquone-proguanil combination is very safe and acceptable. This is because though the intention to treat analysis showed a significant difference of effectiveness from the efficacy, the reasons were not directly related to the combination.

This notwithstanding, however the tolerability of atovaquone and proguanil combination in terms of gastrointestinal side effects requires further clarification in studies in which a fixed dose combination tablet is administered. Reduction in dosage form may hold further benefits and this may have to be looked into in future evaluation and studies.

5.0 CONCLUSION

The re-emergence of malaria and the progressive antimalarial drug resistance represent threats to inhabitants of malaria endemic areas. These underscore the need for new and effective strategies for the multi-drug resistant malaria, although no such satisfactory strategy has yet been established. Indications are that the emergence of drug resistance can be slowed or prevented altogether by use of combinations of antimalarials with different modes of action. However like any other solution there are also difficulties and limitations. The immediate ones are drug toxicities due to interactions, optimum regimen and end product cost. While no single drug may be able to satisfy all these conditions, atovaquone-proguanil with novel structures can be said to have many of these qualities. Result from this study has shown that this combination is novel with excellent cure rate and safety profile for use in malaria treatment. Also to date recrudescence parasitaemia after treatment with atovaquone-proguanil appears to be rare and resistance has not been an issue in individual patients so far treated with atovaquone-proguanil. This notwithstanding however, widespread use of any antimalarial drug will eventually increase the risk that resistance may occur and spread. Atovaquone-proguanil is also potentially susceptible to rapid development of resistance if deployed alone, due to its relatively long parasite clearance time and the relatively long half-life of atovaquone. There will therefore be the need to combine this fixed dose combination with another anti-malarial drug, preferably an artemisinin derivative, so as to prolong its use and life span. Also, at its current price, the cost of atovaquone-proguanil treatment is very high compared to other similar efficacious drugs. It might therefore be necessary to reserve atovaquone-proguanil as a second or third line drug in malaria therapy in endemic areas for patients who fail

treatment with the existing drugs. In fact, this will be in line with the drug manufacturers' donation program with the task force for child survival and development, to make this highly effective but expensive drug available for to those who need most but are least able to afford it.

This study had used meta-analysis in order to improve the precision of estimates of efficacy and effectiveness, since published studies thus far have suffered from small sample sizes. However there are limitations to the techniques used. It must be stated that whilst every effort was made to vigorously search, select and include all studies on atovaquone plus proguanil for malaria treatment, some data might not have been indexed, leading to publication bias. Also it was not possible to acquire the raw data and published literature had to be used. These findings have demonstrated good efficacy, novel mechanism of action, synergistic activity against malaria parasites and favourable adverse effects of the two drugs in patients with *Plasmodium falciparum* malaria. It can be concluded that the above results provide substantial evidence that the atovaquone plus proguanil is safe and effective for *Plasmodium falciparum* malaria treatment. The atovaquone proguanil combination is therefore an important new alternative for *Plasmodium falciparum* malaria and should serve as an important therapeutic option in areas of the world where malaria drug resistance is a problem.

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THANK YOU.