

## Research Article



## COMPARISON OF EFFICACY OF CHLOROQUINE ALONE, AZITHROMYCIN ALONE, AND CHLOROQUINE AZITHROMYCIN COMBINATION FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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### ABSTRACT

To compare the efficacy of chloroquine alone, Azithromycin alone and Azithromycin chloroquine combination as treatment of uncomplicated *plasmodium falciparum malaria*. In this study 50 cases of uncomplicated *falciparum malaria* between 15-75 years of age were studied, irrespective of race and sex. Patients were hospitalized for 7 days and weekly follow up was done for 28 days. Patients were randomly divided into three groups. Group A (16 patients) was given Tab. chloroquine 1500 mg orally for 3 days. Group B (17 patients) was given Tab Azithromycin 1000 mg orally for 3 days and in Group C (17 patients) combination therapy was given. Scoring of the clinical symptoms was done every day during hospital stay and on follow-up weekly for 28 days. Peripheral smear for Malaria Parasite was made every day till 7 days and thereafter on 14th, 21<sup>st</sup> and 28th day. There was rapid defervescence of fever after azithromycin therapy on day 1 & day 2. Same results were observed in chloroquine group and in combination therapy (azithromycin + chloroquine) group. From the present study it can be concluded that, the Azithromycin is a safe and effective drug for the treatment of uncomplicated *plasmodium falciparum malaria* but combination therapy (Tab. Chloroquine + Tab. Azithromycin) was better as compare to Azithromycin and chloroquine respectively because of its rapid clearance of symptoms and no serious adverse effects.

**Keywords:** Azithromycin, Chloroquine, uncomplicated plasmodium falciparum malaria.

### INTRODUCTION

Malaria is a protozoal disease caused by infection with parasite of the genus plasmodium and transmitted to man by four species of infected female. Anopheline mosquitoes of these are *Plasmodium vivax*, *P. ovale*, *P. falciparum* and *P. malaria*, of these *plasmodium falciparum* remains the world's most devastating human parasite infection. Malaria is clinically characterized by typical attacks of three distinct stages- i.e. Cold stage, Hot stage & Sweating stage.

Chloroquine has been the first-line treatment of malaria in much of the world for most of the past 60 years. Although still a first-line treatment for *P. vivax*, chloroquine is no longer recommended for treatment of *P. falciparum* due to high levels of resistance. So, in this study we compared the efficacy of Azithromycin relative to chloroquine as treatment of *plasmodium falciparum malaria*.

Azithromycin is a slow-acting anti-malarial macrolide<sup>1</sup>, an analogue of erythromycin with a nitrogen atom inserted into the macrolide nucleus. As a result, there is enhanced penetration of drug into macrophages, fibroblasts and polymorphonuclear neutrophils, permitting greater accumulation within acidified vacuoles and extending the 1.5-hour half-life of erythromycin to 68 hours for azithromycin<sup>2</sup>. Stable at gastric pH, azithromycin has an absolute bioavailability of 37% following oral administration<sup>3</sup>. It accumulates in hepatic, renal, pulmonary and splenic tissue<sup>4</sup>, and gradually reaches into

the bloodstream over a period of one week. Azithromycin targets the 70 S ribosomal subunit of the apical complex of susceptible micro-organisms including *P. falciparum* and *P. vivax*<sup>5</sup>.

#### Efficacy of azithromycin against malaria

The *in vitro* anti-malarial activity of azithromycin increases 200-fold against *P. falciparum* isolates when incubated between 24 and 48 hours, while its 50% inhibitory concentration values drop as low as 35 nanomolar<sup>6</sup>. At 48-hours, azithromycin is 10-fold more active than erythromycin against chloroquine-resistant *P. falciparum*; the two compounds are equipotent, however, when chloroquine-sensitive parasites are exposed to the same drug concentration<sup>7,8</sup>.

One trial has previously assessed azithromycin as prophylaxis against malaria. Azithromycin was administered daily (250 mg) or weekly (1 g) for 13 weeks to Kenyan adults. The reported symptoms were similar to those of placebo recipients, and there was no evidence of toxicity on routine hematological or biochemical testing<sup>9</sup>.

#### Efficacy of azithromycin and chloroquine combination against malaria

An additive effect between azithromycin and chloroquine has been shown in sensitivity testing conducted over a 48-hour period. When incubation is extended to 68 hours, drug synergy has been seen against chloroquine-resistant isolates; the combination remains additive, however, against chloroquine-sensitive parasites<sup>10</sup>. Sidhu *et al* in



contrast, observed an additive effect at 96 hours of incubation against chloroquine-resistant isolates<sup>11</sup>.

Azithromycin and chloroquine do not exhibit any clinically relevant pharmacokinetic interactions<sup>12</sup>, although chloroquine resistance is reversible with calcium channel blockers, such as verapamil and desipramine, that inhibit p-glycoprotein-mediated efflux<sup>13,14</sup>. Azithromycin is a p-glycoprotein substrate<sup>15</sup> which may suggest the presence of a metabolic mechanism for synergy.

### Aims and objectives

1. To study the efficacy of Azithromycin in the treatment of plasmodium falciparum malaria.
2. To compare the efficacy of Azithromycin relative to chloroquine as treatment of plasmodium falciparum malaria.
3. To assess the efficacy of combination of Azithromycin and chloroquine in treatment of plasmodium falciparum malaria.

### Study area

The study was carried out in the Department of Medicine with collaboration of department of Pharmacology at Netaji Subhash Chandra Bose Medical College, Jabalpur, India during 2002- 2003.

### Selection of cases

- Study was conducted on 50 cases of uncomplicated falciparum malaria patients in the age group 15 to 65 years, irrespective of race and sex. Written informed consent was obtained from each patient. Patients were hospitalized for 7 days and weekly follow up was done for 28 days.
- Patients with serious complications of falciparum malaria, like cerebral malaria, hepatic & renal failure, pulmonary diseases were excluded from the study.
- Patients who were having other diseases (e.g. Tuberculosis, Diabetes, Hepatic, Renal Pulmonary, Cardiac etc.) along with falciparum malaria were also excluded from the study.

## MATERIALS AND METHODS

Patients of plasmodium falciparum malaria were randomly divided into three groups.

- Group A (16 patients) was given Tab. chloroquine 1500 mg orally for 3 days.
- Group B (17 patients) was given Tab Azithromycin 1000 mg orally for 3 days.
- In Group C (17 patients) combination therapy was given.

Chloroquine was given in dose of 1500 mg orally over 72hrs.

Azithromycin was given in dose of 1000 mg daily x 3 days.

- On hospitalization patients clinical evaluation was done meticulously on admission and there after everyday during hospital stay and latter on at follow-up visits.
- Scoring of the clinical symptoms was done every day during hospital stay and on follow-up weekly for 28 days.
- Peripheral smear for Malaria Parasite was made every day till 7 days and thereafter on 14th, 21<sup>st</sup> and 28th day.
- Hematological test which includes - Haemoglobin, total differential counts ESR were done on admission and at 7thday.
- Blood Biochemistry which includes Blood sugar, Blood urea, Serum creatinine, Serum bilirubin and SGOT and SGPT were performed on admission on 7thdays of treatment.
- On admission routine X-Ray chest, Electrocardiogram and Fundus examination were performed.
- Hematological test and blood biochemistry could not be done on follow-up dates on out patients basis as patients did not agree to repeated blood investigations.
- Only peripheral smear for Malaria Parasite was done regularly during hospital stay x 7 days in their after on weekly follow-up to 28 days, along with clinical evaluation of the patient. Parasite count was performed in each slide. Parasite clearance time in hours (PCT) was recorded (PCT = The time taken for parasite count to decrease below the level of microscopic detection).
- Temperature, Pulse, Blood pressure, Checking was done 12 hrly. and PCT (Fever clearance time in hours) was recorded (FCT - is the time taken for fever to return to normal for at least for 24 hours).
- Spleen size regression in Days was recorded.

### Technique used for the detection of malaria parasite in the peripheral blood smear

#### (a) Preparation of the smear

The tip of the middle or ring finger of the left hand was cleansed with spirit swab and allowed to dry completely before pricking. Sharp pricking needle with a triangular cutting edge was used. Two drops of blood were collected, one for the thin smear and one large drop for the thick smear, on the same slide.

#### Thick Smear

It is a smear which transmits enough light for microscopic examination when hemoglobin is partly or wholly removed.

An ideal thick film should be:



- |                                       |      |  |
|---------------------------------------|------|--|
| 1. 10mm away from the edge            | +    | = 1-10 Parasites per 100 thick film fields         |
| 2. 10 mm in Diameter                  | ++   | = 11-100 Parasites per 100 thick film fields       |
| 3. Have 10 layers of RBC              | +++  | = 1-10 Parasites per one thick film fields         |
| 4. Have 10 W.B.C. per immersion field | ++++ | = More than 10 Parasites per one thick film fields |
5. The distance between thick smear and thin smear should preferably be 0.75 cms.

### (b) J.S.B. Stainine

Staining was done' Jaswant Singh & Bhattacharji. The method of staining is as follows.

- The thin smear is fixed with methyl alcohol and on complete drying it is stained with J.S.B. stain care should be taken so that no methyl alcohol is allowed to come in contact with thick smear.
- Immerse the thick and thin smear in J.S.B. stain No. I for 30 seconds.
- Wash it twice or thrice in a Jar containing buffered water for 10 seconds.
- Stain in J.S.B. stain No. II for one second.
- Wash it twice or thrice with buffered water and then dry it in the air.

### (c) Microscopic examination was done to

(I) Identify the species and stage of parasite

(II) Parasite count in per micro liter.

- After staining and drying, the slides were examined with the help of 100x oil objectives and 10 x eye pieces to get the best effect. Before counting begins 100 fields were examined in a thick and thin film to identify the species and the stage of the Parasite.
- Parasite count was done in relation to predetermined number of leukocytes. An average of 1000 leukocytes per micro litre as standard. Then parasite count in relation to leukocyte count converted to parasite per micro litre by the simple mathematical formula.

$$\frac{\text{No. of Parasites} \times 8000}{\text{No. of Leukocytes}} = \text{Parasites per micro litre}$$

For designation of the relative parasite density the following criteria is used in N11EP (for a magnification of 500x to 600x)

- 1-10 Parasite pre 100 thick film fields - **Scanty infection**
- 11-100 Parasite per 100 thick film fields - **Moderate infection**
- More than 10 Parasites per thick film fields - **Heavy infection**

A more simplified method of enumerating parasites in thick blood film is to use plus system. (Essential Malariology- 1st edition)

Thick smear is helpful to detect the parasite quickly because of the large number of parasites seen in each field as compared with thin but due to destruction of R.B.C. in thick smear, species detection of plasmodium requires thin smear examination.

## RESULTS AND DISCUSSION

In this study 50 cases of uncomplicated falciparum malaria between 15-75 years of age were studied. Patients below 15 years of age were not included because this study was conducted in Department of medicine, N.S.C.B. Medical College and Hospital, Jabalpur.

Table 1 shows effective period of Tab. Chloroquine 1500mg for 3 days in 16 patients and Tab azithromycin 1000 mg for 3 days in 17 patients and combination therapy (Tab. Chloroquine + Tab. azithromycin) for 3 days in 17 patients.

Table 2 shows an incidence of various symptoms on admission that is prior to medication. Total 21 symptoms were studied, out of which only 12 symptoms were present. The most frequent symptoms of these patients were headache (92%), fever (88%), rigors (88%), generalized weakness (80%), loss of appetite (44%).

Among less common symptoms were abdominal pain (20%), nausea (36%), vomiting (36%), dry mouth (32%) insomnia (24%), diarrhoea (20%), dizziness (16%).

Table 3 shows 28 patients were between 15-35 years of age, 14 cases were between 36-55years, 6 patients were between 56-65 years of age and 2 patients were between the age group of 66-75 years.

Table 4 shows there were 18 male and 10 female patients in the 15-35 years of age while in the age group 36-55 years were 12 patients male and 2 patients were female and in the age group 56-75 years, 8 patients were male only.

Table 5 shows mean temperature  $104.2 \pm 1.43$  F on admission in group A. Mean temperature on day 1 (24 hrs) was  $(10.2 \pm 1.41)$  and on day 2 (48 hrs)  $(100.14 \pm 0.97)$ . These results reflect rapid defervescence of fever after azithromycin therapy in group A.

Table 6 shows mean temperature  $102.1 \pm 1.41$  F on admission in group B. Mean temperature on day 1 (24 hrs) was  $(100.13 \pm 0.95)$  and on day 2 (48 hrs)  $(98.78 \pm 0.87)$ . These results reflect rapid defervescence of fever after chloroquine therapy in group B.

Table 7 shows mean temperature  $101.89 \pm 1.32$  F on admission in group C. Mean temperature on day 1 (24 hrs) was  $(100.14 \pm 0.94)$  and on day 2 (48 hrs)



(98.72±0.82). These results reflect rapid defervescence of fever after combination therapy in group C.

This study has been established the efficacy of Azithromycin equivalent to chloroquine in the treatment of uncomplicated plasmodium falciparum malaria.

Because of its quick clearance of symptoms and no serious adverse effects it may be used safely in the treatment of uncomplicated plasmodium falciparum malaria.

**Table 1:** Drug wise distribution of uncomplicated *falciparum malaria*

Group	Drug Given	Duration (No. of Days)	No. of Cases
A	TAB. Chloroquine 1500 mg Divided Doses	3 Days	16
B	Azithromycin 1000 mg	3 Days	17
C	TAB. Chloroquine + TAB. Azithromycin	3 Days	17
Total No. of Cases (n) =			50

**Table 2:** Incidence of various symptoms in uncomplicated *falciparum malaria* prior to medication

S. No.	Symptoms	No. of Cases	Percentage
1	Headache	46	92
2	Fever	44	88
3	Vomiting	18	36
4	Rigors	44	88
5	Weakness	40	80
6	Abdominal Pain	10	20
7	Nausea	18	36
8	Loss of Appetite	22	44
9	Dry Mouth	16	32
10	Insomnia	12	24
11	Diarrhea	10	20
12	Dizziness	80	16
Total No. of Cases (n) = 50			

**Table 3:** Age wise distribution of uncomplicated *falciparum malaria*

S. No.	Age	No. of Cases	Percentage
1	15-25	18	36
2	26-35	10	20
3	36-45	10	20
4	46-55	4	8
5	56-65	6	12
6	66-75	2	4
Total No. of Cases (n) = 50			

**Table 4:** Age and sex wise distribution of uncomplicated *falciparum malaria*

S. No.	Age	Male	Female
1	15-25	10	8
2	26-35	8	2
3	36-45	8	2
4	46-55	4	0
5	56-65	6	0
6	66-75	2	0
Total No. of Cases (n) = 50		38	12

**Table 5:** Effect of Azithromycin on clinical parameters of group A

Parameters	Days							
	D0	D1	D2	D3	D4	D7	D14	D28
Body Temperature °F	104.2±1.43	102.2±1.41	100.14±0.97	98.76±0.89	98.38±0.68	97.28±0.39	98.26±0.44	98.22±0.32
Pulse / Min.	99.86±12.18	90.12±14.16	88.12±10.12	86.4±9.40	80.66±6.42	80.62±6.52	78.64±6.32	76.2±4.58
Blood Pressure Syst. (mm of Hg)	113.32±12.58	114.24±11.12	111.84±9.40	113.6±7.66	111.44±7.16	112.76±7.9	112.48±8.59	111.34±6.79
Blood Pressure Diastolic (mm of Hg)	71.4±8.04	70.4±7.16	113.6±7.66	71.10±7.34	71.32±5.88	7.24±5.06	73.14±5.05	74.16±6.40



**Table 6:** Effect of Chloroquine on clinical parameters of group B

Parameters	Days							
	D0	D1	D2	D3	D4	D7	D14	D28
Body Temperature °F	102.1±1.41	100.13±0.95	98.78±0.87	97.37±0.38	98.26±0.42	98.43±0.37	98.22±0.43	98.34±0.12
Pulse / Min.	99.84±14.18	88.14±12.16	84.6±9.42	80.34±6.46	80.4±6.56	76.54±4.14	78.62±6.34	77.4±4.62
Blood Pressure Syst. (mm of Hg)	114.34±14.56	113.24±10.16	111.82±7.24	113.44±7.64	112.40±8.14	112.74±7.10	132.44±8.56	12.32±7.78
Blood Pressure Diastolic (mm of Hg)	70.2±8.02	70.2±7.12	72.24±6.56	71.10±7.30	72.33±5.92	74.20±5.09	73.12±5.70	74.14±6.38

**Table 7:** Effect of combination therapy on clinical parameters of group C

Parameters	Days							
	D0	D1	D2	D3	D4	D7	D14	D28
Body Temperature °F	101.89±1.32	100.14±0.94	98.72±0.82	98.40±0.80	90.40±0.78	98.42±0.68	98.34±0.36	98.32±0.36
Pulse / Min.	98.82±12.14	88.14±10.10	84.4±9.40	78.64±7.40	79.4±7.57	80.2±6.56	76.54±4.18	79.68±6.32
Blood Pressure Syst. (mm of Hg)	112.34±14.50	113.28±12.12	111.24±8.26	112.2±6.66	110.48±8.16	112.78±7.18	113.48±8.54	113.49±8.48
Blood Pressure Diastolic (mm of Hg)	70.6±8.04	72.2±8.03	71.4±7.13	71.6±6.58	72.38±6.54	74.22±5.08	73.10±4.70	72.12±4.71

## CONCLUSION

The emergence and spread of drug resistant strains of *plasmodium falciparum* constitutes a serious problem in management of uncomplicated malaria.

Chloroquine resistance has been reported from different parts of the world and resistance to quinine has been observed in Thailand and Vietnam (WHO: 1987). The problem is aggravated by a scarcity of quinine in many regions.

There is thus an urgent need to find an effective antimalarial drug with minimal adverse effects. Azithromycin has opened up new horizons in the treatment of uncomplicated *falciparum malaria* (Am J Trop Med Hyg, 1992; 47 (3): 378-82.) (Hyg 1995, 52(2): 159-61.)

In this study azithromycin was administered to 17 patients with uncomplicated *falciparum malaria* in a dose of 1000 mg orally over 3 days.

Chloroquine was administered to 17 patients with uncomplicated *falciparum malaria* in a dose of 1500 mg (in divided doses over 3 days).

Combination therapy of (Tab. Chloroquine + Tab. Azithromycin) was given to 17 patients over 3 days.

Most of the patients became afebrile within 48 hours and all of the cases were afebrile by 72 hours.

No significant effect on Haematological and biochemical parameters was found. No adverse effect was seen. In our study Azithromycin was found safe, effective and convenient than chloroquine for treating uncomplicated malaria but combination therapy (Tab. Chloroquine + Tab. Azithromycin) was better as compare to Azithromycin and chloroquine respectively because of its rapid clearance of symptoms and no serious adverse effects.

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