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# Prevalence of chloroquine resistant Plasmodium falciparum malaria in pregnant females attending North Indian hospital

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**ABSTRACT**

Chloroquine resistance was first reported in South-east Asia and South America region and has now spread to the vast majority of malaria endemic countries. P. falciparum malaria is a cause of high morbidity and mortality in general population as well as in antenatal females.

1. To evaluate the accuracy of different diagnostic tests for diagnosis of malaria in pregnancy.
2. To determine the level of chloroquine resistance in P. falciparum isolates from pregnant females.

The study was conducted in Department of Microbiology and Obstetrics and Gynaecology of Jawaharlal Nehru Medical College and Hospital. A total of 156 pregnant females were included in the study. Diagnosis of malaria was done by blood smear examination or antigen detection assay or by Quantitative Buffey Coat assay. P. faciparum was cultured in RPMI 1640 medium and in-vitro drug sensitivity was done by microtest-II WHO sensitivity plates against chloroquine.

P. falciparum culture was positive in 66 pregnant females. 28 isolates were found to be resistant to chloroquine. The effective concentration of chloroquine EC 50 was found to be 6.6, EC90 34.3, EC95 45.4, EC99 52.1 nmol/l.

High level of chloroquine resistance in pregnant females can lead to serious complications in antenatal patients and their fetuses.

**Key Words:** Chloroquine resistance, in-vitro drug sensitivity, effective concentration

## Introduction

Malaria is an important public health problem in countries where its transmission occurs regularly, as well as in areas where transmission has been largely controlled or eliminated. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where it had been eradicated. P. falciparum has developed resistance to nearly all antimalarials in current use.

Chloroquine, inexpensive 4-amino quinolone compound has been used for decades as primary drug in malaria treatment. This accumulates inside the digestive vacuoles of the infected RBCs. Chloroquine resistance was first reported in South-east Asia and South America and has now spread to the vast majority of malaria endemic countries (1). Resistance of P. falciparum to chloroquine is believed to be due to an increased capacity of the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of haem polymerization (2). This chloroquine efflux occurs at an increased rate of about 40 to 50 times faster among resistant parasites than sensitive strains (3). This test reflects pure antimalarial drug resistance.

Prevalence of malaria is higher among pregnant women than other groups (4) and that this can lead to abortion, intrauterine fetal death, premature delivery and even maternal death (5). Pregnant females are prone to hypoglycemia, acute pulmonary edema, hemolytic anemia, fetal distress, premature labor and stillbirth (6). The problem of antimalarial drug resistance is greater for pregnant women because of their increased risk of malaria (7) and the limited availability of anti-malarial drugs that can be used in pregnancy (8). Moreover, there occurs physiological changes in pregnancy that can alter drug metabolism (9)

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particularly in the placenta, where parasites accumulate in high densities (10).

Although chloroquine is the treatment of choice in pregnant females, chloroquine-resistant strains of P. falciparum are spreading rapidly (11) and when a pregnant woman is infected with chloroquine-resistant P. falciparum, the alternative choice of drugs must consider potential adverse effects to the fetus and mother (12). Thus to supplement the previous studies and to find out the prevalence of chloroquine-resistant strains of P. falciparum among pregnant females the present study was undertaken with following objectives:

1. To evaluate the accuracy of different diagnostic tests for diagnosis of malaria in pregnancy.
2. To determine the level of chloroquine resistance in P. falciparum isolates from pregnant females.

## Material and methods

The study was conducted in Department of Microbiology and Obstetric and Gynaecology of Jawaharlal Nehru Medical College and Hospital, Aligarh. A total of 156 pregnant females having symptoms suggestive of severe malarial infection who were admitted in obstetric wards were included in the study.

**Specimen collection:** P. falciparum parasite isolates cultivated *in vitro* followed modification of the standard culture techniques (13), while drug susceptibility test followed the standard procedure for schizont inhibition (14). About 5mL of blood was collected by veinpuncture for culture after taking all sterile precaution and transferred to a heparinized centrifuge tube and stored at 4ºC and was transported in ice to the laboratory (14). Both thick and thin smears were examined after staining with giemsa stain. Diagnosis of malaria was done using different diagnostic tests like blood smear examination or by QBC assay or antigen detection assay.

**Culture and sensitivity:** The culture medium consisted of RPMI 1640 (contains HEPES buffer), 2 g glucose, and 40 µg/mL gentamycin sulphate (to avoid contamination) with 10% AB+ serum. Culture medium was sterilised by filtration through a millipore filter. The drug sensitivity can be determined *in vitro* by using standard 96-well microtitre plates (WHO plates). The test plates were predosed with increasing concentrations of chloroquine A–H 0, 1, 2, 4, 8, 16, 32, and 64 pmol. Well A was the control. Blood medium mixture (BMM) was prepared by shaking the tube. BMM is stable for several hours. Preculture thick and thin films were taken from suspected persons and stained with Giemsa or another reliable Romanosky stain. All the wells were dosed with 50 µL of the blood medium mixture (1:9) using Effendorf pipette and a disposable sterile tip. Dosing was always done starting with control well (A); following an increasing order of concentrations, ending with well H. The plate was shaked gently so as to dissolve the deposits. The plate was put in a candle jar in the incubator at 37.5ºC for 24-30 hrs. After 24-hour incubation, thick smears were prepared from each well (from the red blood cells deposited at the bottom of the well) after removing the supernatant with a micropipette. For an acceptable test, schizont maturation in control well (A) must be 10% or more (20 schizonts with 3 or more nuclei per 200 asexual parasites). Counts in the drug wells were expressed as % of control. For chloroquine, satisfactory response is said if there is complete schizont inhibition at 4 mol or less. If there is schizont formation at 8 pmol or more, it is an indication of resistance.

**Determination of *in vitro* effective concentration (EC) values of the chloroquine:** The mean number of schizonts count per well was fed directly into nonlinear regression software. Individual dose response curves were generated and their EC 50, EC 90, EC 95 and EC 99 values determined.

**Data Analysis:** The geometric means and 95% confidence intervals (CIs) of EC values were estimated in SPSS 17. The study had been approved by the "Institutional Ethics Committee" of the faculty of Medicine, Jawaharlal Nehru Medical College and Hospital, Aligarh.

## Results

Out of 156 females included in the study, 89 females had P. falciparum infection as diagnosed by blood smear examination, QBC and antigen detection assay. Out of 89 females diagnosed as cases of P. falciparum malaria, 13(14.6%) cases were found to be positive by MP smear, 72(81%) by QBC and 89(100%) by RDT.

Fever and chills were present in all patients; rigors in 73%, anaemia in 80%, hypoglycemia in 35% and altered consciousness was present in 26.9% patients (Table 1).

Blood from 89 patients who were positive for P. falciparum infection was cultured. P. falciparum

was isolated from 66 blood specimens. These isolates were subjected to antimalarial sensitivity test using WHO *in vitro* micro-test kit sensitivity plates. *In vitro* sensitivity testing against chloroquine showed 28 isolates to be resistant to chloroquine (Figure 1). Thus the prevalence of chloroquine resistance was found to be 31.5%. This is schizont maturation at 8 pmol of drug. Some isolates showed maturation upto 36 pmol or more. The effective concentration of chloroquine EC 50 was found to be 6.6, EC90 34.3, EC95 45.4, EC99 52.1nmol/l (Table 2).

**Table 1.** Clinical features of P. falciparum infection

| S.No | Clinical features | No of patients (89) |
|---|---|---|
| 1 | Fever | 89 (100%) |
| 2 | Chills | 89 (100%) |
| 3 | Anaemia | 71 (80%) |
| 4 | Rigors | 65 (73%) |
| 5 | Hypoglycemia | 31 (35%) |
| 6 | Altered consciousness | 24 (26.9%) |
| 7 | Convulsions | 15 (16.8%) |
| 8 | Neck rigidity | 10 (11.2%) |
| 9 | Other signs of meningeal irritation | 8 (8.9%) |
| 10 | Yellowish discoloration of sclera | 2 (2.2%) |
| 11 | Renal complications | 0 |

**Table 2.** Geometric mean EC50, EC90, EC95 and EC99 of chloroquine against P. falciparum isolates

| Chloroquine | Geometric mean EC, (95% CI) nanomolar (nmol) | |
|---|---|---|
| | Sensitive isolates n= 61 | Resistant isolates n= 28 |
| EC 50 | 1.30 (1.0-1.99) | 6.6 (2.74-13.42) |
| EC 90 | 3.94 (1.88-5.53) | 34.3 (16.94-61.09) |
| EC 95 | 5.56 (2.48-7.22) | 45.4 (19.15-65.78) |
| EC 99 | 6.17 (2.8-7.83) | 52.1 (21.39-66.98) |

EC- Effective concentration

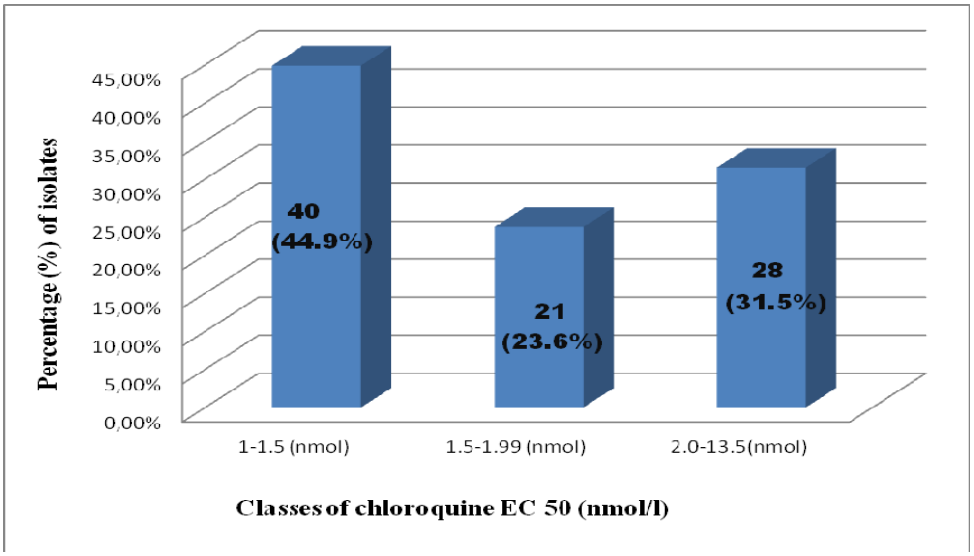


**Fig. 1.** Relative EC 50 (nmol) distribution pattern of chloroquine among P.falciparum isolates.
EC- Effective concentration

## Discussion

Some population groups are at considerably higher risk of contracting malaria and suffering from, or dying of it, than others. They include pregnant women, patients with HIV/AIDS, non-immune travellers, children and under five years of age in high transmission areas. They warrant particular measures for prevention of malaria and to mitigate this risk, taking into consideration their specific circumstances and the tools and strategies available. High level of chloroquine resistance in P. falciparum in this area can lead to serious complications in antenatal patients and their fetuses. In pregnant women there are more chances of developing high parasitemia with anemia, hypoglycemia (11) than non pregnant females. These complications were also observed in study group. Due to the hormonal and immunological changes, the parasitemia tends to be 10 times higher and as a result, all the complications of falciparum malaria are more common in pregnancy compared to the non-pregnant females. The disease results from the aggregation of erythrocytes infected by P. falciparum which have been shown to adhere to chondroitin sulfate A (CSA) on placental proteoglycans causing them to accumulate in the intervillous spaces of the placenta, blocking the crucial flow of nutrients from mother to embryo (11).

In areas endemic for malaria, highest risk for infection and morbidity is in primigravidas, adolescents, and those coinfected with HIV (15). Similarly maximum number of patients (57.2%) were primigravidae in this study.

Although chloroquine is the treatment of choice in pregnant women but there are reports of emergence of chloroquine resistance in India (16) and worldwide (18). On *in vitro* antimalarial sensitivity testing chloroquine resistance was observed in 28 isolates. High level of chloroquine resistance in general population have been reported by us in 2012 (17). There are limitations for use of other drugs in pregnancy as mefloquine is contraindicated in pregnancy (11) and Halofantrine although effective against chloroquine-resistant strains of P. falciparum, but data is insufficient to permit its use in pregnancy (12). In a situation like this, quinine seems to become the drug of choice (11). Quinine shows no increased teratogenic risk and no risk of premature labor; but in pregnant females, in particular, are at risk of quinine-induced hyperinsulinemia. In Quinine resistant strains artemisin derivatives, artemether and artesunate can be used (19).

In our study out of 156 females, 89 (57%) had P. falciparum infection. This is in corroboration with findings of Bouyou-Akotet et al. (20) who conducted a study in Gabon. In a study conducted in Kenya by Rukaria-Kaumbutho et al. (17) P. falciparum infection was found in 65 patients out of 300 (22%).

This high level of resistance may be due inadequate treatment, improper prescribing habits of quacks, spurious medications and due to acquisition of resistant strains present in this area. This pattern of chloroquine resistance is in accordance with our previous published work (18) which showed schizont maturation upto 36 pmols or more. We cannot compare our study of *in vitro* sensitivity testing of chloroquine resistance as no such study have been done on pregnant females in India. This is the first study of this type on pregnant females. Various workers have analysed the pattern of chloroquine resistance in general population in other parts of India and observed 48/108 (44.4%) isolates to be resistant to chloroquine (16).

In our study effective concentration 50 (EC50) of chloroquine was found to be 1-1.99 nmol/l for sensitive isolates and very high values upto 13.42 nmol/l for resistant isolates. High EC50 for chloroquine (33.7 nmol/l) was also noted by Anvikar et al. (16). So this high level of resistance needs to be monitored on a regular basis to combat prevailing drug resistance and to prevent the spread of resistant strain far and wide of this geographic region.

A large proportion of pregnant women with malaria do not respond to chloroquine therapy and alternative drugs are therefore required. High level of chloroquine resistance in pregnant females in this area can lead to serious complications in antenatal patients and their fetuses.

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