CHLOROQUINE CONCENTRATION PROFILE IN THE COMMUNITY OF MEWAT REGION, DISTRICT GURGAON (HARYANA), INDIA

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Abstract. A survey was conducted to find chloroquine concentration profile in the community of Mewat region district Gurgaon (Haryana) of India. 88 *P. falciparum* and 3 *P. vivax* cases were detected out of 148 blood slides examined with a SPR of 61.48. Plasma chloroquine and desethylchloroquine concentrations were determined in 55 *P. falciparum* and 2 *P. vivax* patients and 29 persons whose blood slides were negative for malaria parasite before giving any treatment. Mean chloroquine concentrations in cases with *P. falciparum* parasites and without malaria parasites were 0.018 and 0.016 μg ml^{-1} respectively. Chloroquine to desethyl chloroquine ratio was between 2 and 3 in both groups. Only 10 malaria parasite negative cases out of 29 had plasma chloroquine concentrations above 0.016 μg ml^{-1} required for malaria chemoprophylaxis. Chloroquine was undetectable in plasma samples of 8 out of 55 *P. falciparum* cases. Chloroquine plasma concentrations in 21 *P. falciparum* cases were below therapeutically effective concentration of 0.016 μg ml^{-1} suggesting improper treatment while in 29 *P. falciparum* cases, parasitemia recurred despite required chloroquine concentration confirming chloroquine resistant status. Irregular prophylaxis and lack of proper treatment was one of the major causes of malaria outbreak in this area.

INTRODUCTION

About 2 million new malaria cases are reported every year and 40-50% of these cases are caused by *Plasmodium falciparum*. Chloroquine has long been the drug of choice for the treatment and prevention of malaria. However resistance of *P. falciparum* to chloroquine is now widespread in India (Pattanayak *et al*, 1994) and resistance in *P. vivax* has also been reported (Garg *et al*, 1995; Dua *et al*, 1996). Chloroquine resistance is developed due to its irregular use particularly in rural areas. During the transmission season, a subcurative dose of chloroquine is very commonly used for prophylaxis as well as for the treatment of symptomatic malaria cases. Recently there was an outbreak of malaria in the Mewat region of district Gurgaon (Haryana). India during October-November 1996 and about 10,000 cases were recorded by the State Health Office. Chloroquine failure has been reported by local medical practioners. We have surveyed some areas with the aim to find the chloroquine concentration profile in the community.

MATERIALS AND METHODS

Mewat region is situated in Gurgaon district of Haryana which is about 80 km from Delhi. The region is the border area of Rajasthan where an outbreak of malaria occurred in 1995. The surveillance and treatment of malaria was very poor and most of the suspected malaria cases were treated by local medical practioners.

The study was performed in the month of November, 1996. Active surveillance was carried out by the project staff of the Malaria Research Center. Since *P. falciparum* cases were very high inspite of frequent use of chloroquine, all *P. falciparum* cases were treated with 3 tablets of Fansidar (1,500 mg sulfadoxine with 75 mg pyrimethamine) as a single adult dose within 12 hours of the blood slide collection.

Venous blood (2.5 ml) was drawn from each case at the time of slide collection and kept in heparinized vials. Plasma samples were obtained by centrifugation at 1,000*g* for 20 minutes and kept at -20°C until analysis.

All samples were analysed for chloroquine and desethylchloroquine using the HPLC method described by Alvan *et al* (1982). In brief, acetonitrile-methanol-diethylamine (60:4:0.5, v/v) was used as a mobile phase on a μPorasil column (30 x 4.6 mm) at 1.00 ml $minute^{-1}$. The detection was carried out with a fluorescence detector operated at Em 390 and Ex 350 nm. The internal standard was 4-(4-dimethylamino-1-methylbutylamino)-7-chloroquinoline. The lower limit of sensitivity was 1 ng ml^{-1} for chloroquine and 0.5 ng ml^{-1} for desethylchloroquine.

RESULTS AND DISCUSSION

A total of 148 blood slides were collected for the examination of malaria parasites, of which 91 cases were found positive [slide parasite rate (SPR) =61.48] with 88 cases of *P. falciparum* [slide *falciparum* rate SFR = 59.45]. Plasma samples for chloroquine determination were obtained from 86 cases (51 males and 35 females). 55 and 2 plasma samples were from *P. falciparum* and *P. vivax* patients respectively while 29 samples were from malaria negative cases. Other persons did not turn up or refuse to give blood.

Mean chloroquine and desethylchloroquine concentrations in *P. falciparum* cases and malaria parasite negative cases are given in Table 1.

Chloroquine and desethylchoroquine concentrations in malaria parasite negative cases

The mean age of 29 malaria parasite negative cases was 36 years. Chloroquine was detected in 26 out of 29 cases under study. Chloroquine concentrations ranged from ND-0.08 μg ml^{-1} with a mean value of 0.016 μg ml^{-1}. In one case, the chloroquine concentration was very high (0.23 μg ml^{-1}) and it was not included in the calculation of the mean value. Similarly desethylchloroquine concentrations ranged from ND-0.04 μg ml^{-1} with a mean value of 0.005 μg ml^{-1}.

A large variation was recorded between the different samples which was much higher than the inter-individual difference recorded earlier (Hellgren *et al*, 1989). Ten cases had chloroquine concentrations above targeted concentration of 0.016 μg ml^{-1} to suppress malaria parasites (Bruce-Chwatt 1981) while 19 cases had either nil or lower concentration than required for malaria chemoprophylaxis. This implies that mostly this group of the population either did not take chloroquine or they were irregular takers with low dose. It may be noted that chloroquine prophylaxis is not common in rural area and symptomatic cases are treated generally with chloroquqine by medical practioners. Recently Wetsteyn and coworkers (1995) have reported that a chloroquine concentration of 0.016 μg ml^{-1} is sufficiently suppressive for malaria chemoprophylaxis. The chloroquine to desethylchloroquine concentration ratio was 2 to 3 times which is in agreement with the earlier reports (Hellgren *et al*, 1989).

Chloroquine and desethylchloroquine concentrations in *P. falciparum* malaria cases

The average age of 55 *P. falciparum* cases was 25 years while their average weight was 46 kg. The median density before treatment was 3,136 μl^{-1} (range 1,760-5,280 μl^{-1}). All slides had rings stage while some slides contained rings with gametocytes.

Chloroquine concentrations in 8 cases were below the detection limit of 0.2 ng ml^{-1}. In 3 cases chloroquine concentrations were very high as compared to other cases and were not included to determine mean chloroquine levels. The chloroquine concentrations of these cases were 0.10, 0.12 and 0.24g ml^{-1}. Mean chloroquine and desethylchloroquine concentrations in 52 *P. falciparum* cases were 0.018 μg ml^{-1} (range ND-0.07 μg ml^{-1}) and 0.008 μg ml^{-1} range (ND-0.03 μg ml^{-1}) respectively.

Eight cases where chloroquine levels were below detection limit may be considered as non-takers of chloroquine for prophylaxis or symptomatic treatment and categorized as fresh cases. In 21 *P. falciparum* cases, chloroquine concentrations were

Table 1
Mean chloroquine and desethylchloroquine concentrations in plasma in persons with and without *P. falciparum* malaria.

Cases	Mean age/wt	Concentration (μg ml^{-1})	
		Chloroquine (range)	Desethylchloroquine (range)
Without malaria parasites (n=27)	36/50	0.016 (ND-0.08)	0.005 (ND-0.04)
With *P. falciparum** malaria (n=52)	25/46	0.0186 (ND-0.07)	0.008 (ND-.03)

*Mean parasite density = 3,136 μl^{-1} (range 1,760-5,280 μl^{-1})

below therapeutically effective plasma concentration of 0.016 μg ml^{-1} to kill sensitive *P. falciparum* parasites (Bruce-Chwatt, 1981). The study implied that these persons had taken subcurative symptomatic chloroquine treatment. During personal discussion, it was noticed that all 21 patients had taken chloroquine within one week of sampling. 26 *P. falciparum* cases showed plasma chloroquine concentrations higher than the therapeutic plasma concentration for drug sensitive *P. falciparum* parasites and parasitemia occurred despite required chloroquine concentrations which confirm that these *P. falciparum* cases were resistant to chloroquine. It is to be pointed out that all 26 cases were treated with chloroquine for symptomatic malaria by medical practioners. Aronsson *et al* (1981) have reported a serum chloroquine concentration of 0.06 μg ml^{-1} in two cases of chloroquine resistant *P. falciparum* malaria at the time of re-appearance of parasites while Hellgren *et al* (1989) have found MIC of whole blood chloroquine of 0.25 (range 0.142-0.278) μg ml^{-1} for R II resistant strains and 0.047 (range 0.014-0.215) μg ml^{-1} for parasites that reappeared.

Three cases have parasites in spite of very high plasma chloroquine concentration and should be viewed as cases of RII/RIII level of chloroquine resistance. During personal discussion, it was found that most of the patient had taken chloroquine irregularly depending upon their physical conditions. All 55 *P. falciparum* cases were treated with 3 tablets of Fansidar (1,500 mg sulfadoxine with 75 mg pyrimethamine) and no case of recrudescence occurred.

Chloroquine concentration in *P. vivax* cases

Chloroquine concentration in two *P. vivax* cases were 0.01 and 0.005 μg ml^{-1} respectively. In one case the chloroquine was slightly lower than the required therapeutic concentration while the other case had very low chloroquine concentration which clearly showed lack of proper treatment or prophylaxis. It is to report that a case of chloroquine resistant *P. vivax* malaria was recently detected in adjoining Mathura district (Dua *et al*, 1996).

Our study clearly showed irregular prophylaxis and lack of proper treatment in Mewat region which may be one of the reasons for an outbreak of malaria in this region. All symptomatic malaria cases were treated with chloroquine by local doctors however, over 52.7% *P. falciparum* cases had parasitemia inspite of required chloroquine plasma concentrations, showing evidence of resistance to chloroquine. Therefore, prompt case detection, proper treatment, effective vector control measures and change in drug policy are urgently needed in the area.

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REFERENCES

Alvan G, Ekman L, Lindstrom B. Determination of chloroquine and its desethyl metabolite in plasma, red blood cells and urine by liquid chromatography. *J Chromatogr* 1982; 229: 241-47.

Aronsson B, Bengtsson E, Bjorkman A, Pehrsson PO, Rombo L, Wahlgren M. Chloroquine-resistant malaria in Madagascar and Kenya. *Ann Trop Med Parasitol* 1981, 75: 367-73.

Bruce-Chwatt LJ. Chemotherapy of malaria, 2nd ed. World Health Organization, Geneva, 1981: 68.

Dua VK, Kar PK, Sharma VP. Chloroquine resistant *Plasmodium vivax* malaria in India. *Trop Med Int Health* 1996; 1: 816-19

Garg M, Gopinathan N, Bodhe P, Kshsirsagar NA. *Vivax* malaria resistant to chloroquine: case reports from Bombay. *Trans R Soc Trop Med Hyg* 1995; 89: 656-57.

Hellgren U, Kihamia CM, Mahikwano LF, Bjorkman A, Erikson O, Rombo L. Response of *Plasmodium falciparum* to chloroquine treatment: relation to whole blood concentrations of chloroquine and desethylchloroquine. *WHO Bull* 1989; 67: 197-202.

Pattanayak S, Sharma VP, Kalra NL, Orlov US, Sharma RS. Malaria paradigms in India and control strategies. *Ind J Malariol* 1994; 31: 141-99.

Wetsteyn JCFM, De Vries PJ, Oosterhuis B, Van Boxtel CJ. The pharmacokinetics of three multiple dose reqimen of chloroquine: implications for malaria chemoprophylaxis. *Br J Clin Pharmacol* 1995; 39: 696-9.