6. Kshirsagar, S. G., Patole, M. S. and Shouche, Yogesh S., *In Vitro Cell. Dev. Biol. Animal*, 1998, **34**, 665–667.
7. Kshirsagar, S. G., Patole, M. S. and Shouche, Yogesh S., *Anal. Biochem.*, 1997, **253**, 65–69.
8. Wurster, D. H. and Benirsche, K., *Science*, 1970, **168**, 1364–1366.
9. Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X. and Wilson, A. C., *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 6196–6200.
10. Lee, S. Y. and Rasheed, S., *Biotechniques*, 1990, **9**, 676–679.
11. Maidak, Bonnie, L. *et al.*, *Nucleic Acids Res.*, 2000, **28**, 173–174.
12. Miyamoto, M. M., Kraus, F. and Ryder, O. A., *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 6127–6131.
13. Lang, B. F., Gray, M. W. and Burger, G., *Annu. Rev. Genet.*, 1999, **33**, 351–397.
14. Lan, H., Wang, W. and Shi, L., *Biochem. Genet.*, 1995, **33**, 377–388.
15. Sudhir Kumar, Koichiro Tamura, Ingrid Jakobsen and Masatoshi Nei MEGA: Molecular Evolutionary Genetics Analysis, Version 2.0, Pennsylvania State University, University Park and Arizona State University, Tempe, USA, 2000.

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Status of chloroquine efficacy against *Plasmodium falciparum* in pregnant women in a tribal area of central India

The World Health Organization (WHO) recommended that all pregnant women living in malaria-endemic areas should be given initial antimalarial treatment followed by chemoprophylaxis throughout pregnancy, in an effort to prevent the adverse effects of *Plasmodium falciparum*[1]. In India, chloroquine (CQ) is the drug of choice, though the susceptibility status of *P. falciparum* to CQ in pregnant women is not known. A longitudinal study carried out earlier in Mandla district, Madhya Pradesh (MP) revealed malaria as a major health problem in this area and 21% pregnant women were positive for malaria; 36% were *P. vivax* and 64% were *P. falciparum*. There were no instances of cerebral malaria or death, however anaemia was commonly prevalent in 80% of the women[2]. Hospital-based studies showed that pregnant women with falciparum or vivax malaria were significantly more anaemic than non-infected pregnant women or infected non-pregnant women[3]. CQ failed to cure 8% pregnant women with uncomplicated *P. falciparum* infections[2]. To evaluate the efficacy of CQ (25 mg/kg body weight) in the treatment of *P. falciparum* in pregnant women, this study was initiated in a malaria meso-endemic area of central India.

The study was conducted from October to December 1996 in 10 villages (90% Gond ethnic tribe) in Mandla district. The villages are scattered, thinly populated and without any communication. Villagers are mostly illiterate, poor and work mainly in forest nurseries. In this community deliveries occur at home with the assistance of family members or by traditional birth attendants. Malaria transmission is almost perennial. *P. vivax* is the dominant species from February to June and *P. falciparum* is mainly prevalent from July to January.

Pregnant women (4–7 months) with fever, with a history of fever, headache and joint pains were screened for malaria parasite. Information was obtained from each patient about age, parity and antimalarial drug use. Thick and thin blood films were made from finger pricks and stained with Giemsa. The study protocol was approved by the Institutional Review Committee, Malaria Research Centre (Indian Council of Medical Research, New Delhi). Only pregnant women with asexual forms of *P. falciparum* and with no history of antimalarial drug intake in the previous seven days and whose urine samples were negative for CQ by Dill and Glazko method[4] were eligible for this study after obtaining verbal consent. Women with signs of severity were excluded[5]. The patients were given CQ (25 mg/kg body weight) under supervision in 3 divided doses. *In vivo* test was carried out according to Rieckmann's simplified method[6]. The procedure for classifying the response to treatment is presented in Figure 1. The study group (21) was given insecticide-treated bed-nets with the advise to sleep under these bed-nets.

Information was obtained about fever or vomiting after the drug was taken and about the use of bed-nets and their urine samples were tested again for CQ on day 2. Treatment failure was defined by the presence of asexual forms of *P. falciparum* on day 2 or later in the 4 weeks of follow-up. Early failures were treated with Fansidar (1500 mg sulphadoxine and 75 mg pyrimethamine, SP) and followed weekly for 4 weeks. Patients who became parasitaemic at day 14 or later were given CQ again and also followed for 4 weeks. Patients with asexual parasitaemia after second dose of CQ were treated with Fansidar.

Haemoglobin (Hb) was estimated in field by the haematic acid method, originally described by Sahli[7], before treatment and subsequently after treatment during follow-up on day 7 or 14.

Out of 100 pregnant women screened, 28 were found positive with *P. falciparum* and 2 with mixed infections of *P. vivax* and *P. falciparum*. Twenty-one women were eligible for the study (mean age 26.5 ± 3.5 years; mean weight 47.5

Table 1. Geometric mean parasite density of *P. falciparum* in pregnant women after treatment with chloroquine (25 mg/kg body weight)

	Asexual parasite/mm^3 on days						
Pregnant women	D_0	D_2[a]	D_7[a]	D_{14}[b]	D_{21}[b]	D_{28}[c]	D_{35}[c]
Primigravidae (P_0)	3777.83 ± 6.67 (5)	1116.41 ± 3.02 (3)	0	0	1206.43 ± 14.74 (2)	0	771 ± 0 (1)
Multigravidae (P_1–P_4)	1007.35 ± 4.13 (16)	788.96 ± 4.40 (4)	4510 ± 0 (1)	746.76 ± 5.36 (8)	462.03 ± 2.02 (3)	1029.69 ± 4.90 (7)	1002.94 ± 2.02 (2)

Pooled data ± S.D.
[a]Treated with Fansidar.
[b]Treated with 2nd dose of chloroquine.
[c]Reappearance/recrudescence of parasite after 2nd dose of chloroquine. Treated with Fansidar.
Figures in parentheses indicate the number of patients.

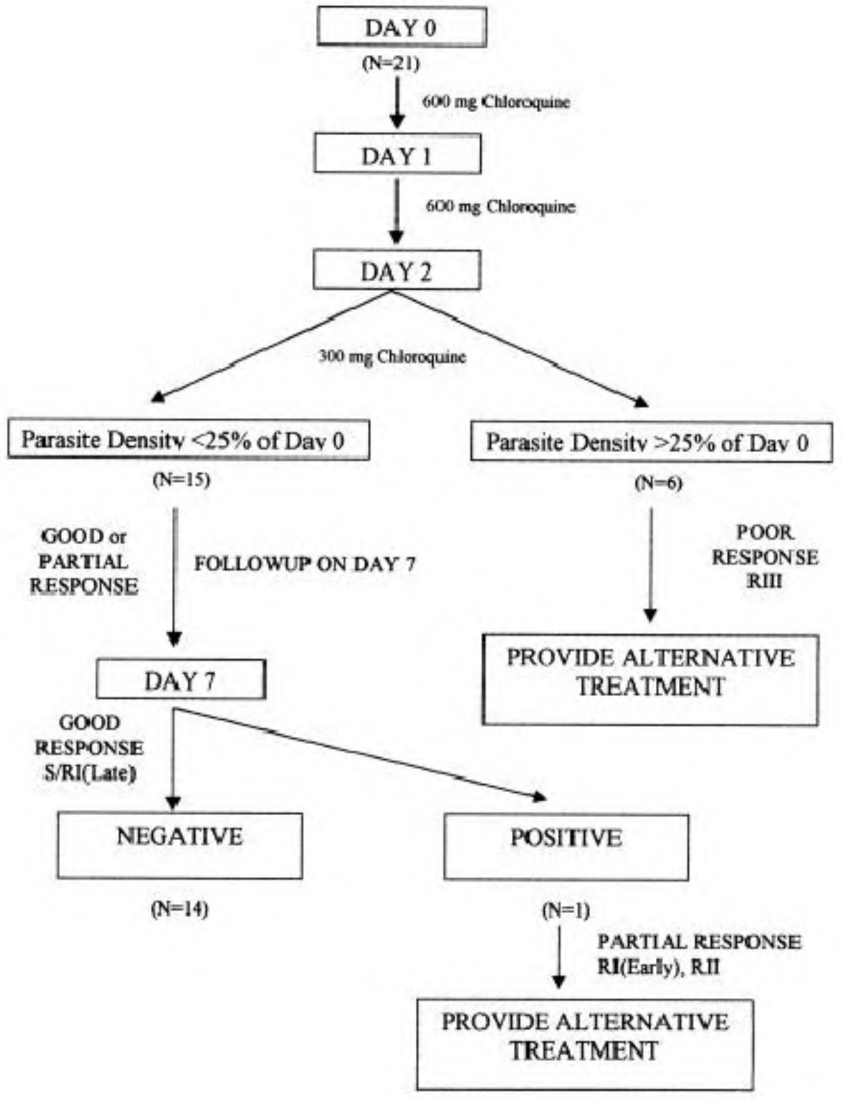

Figure 1. Scheme for monitoring chloroquine resistance in *P. falciparum* (based on the Rieckmann simplified *in vivo* method).

± 6.2 kg). Six (28.6%) women (2 primi + 4 multi) had a RIII type response (95% C.I., 9–48%). Only one (4.7%) multigravid showed partial response (RI early/RII). Remaining (66.7%) women (3 primi + 11 multi) had a late RI/S type of response (Figure 1), out of which only one primigravid appeared to be truly sensitive to CQ. Thus the cumulative failure rate in this study was 95% (95% C.I., 86.13–100%). Out of these 13 women (2 primi + 11 multi) who failed and were treated with CQ, 10 (1 primi + 9 multi) failed (77%) again (95% C.I., 48–95%) on day 28 and day 35 (Table 1). All these women were asymptomatic at the time of recrudescence, may be because of low parasite density. On the contrary, none of the women treated with SP was found to be parasitaemic during the 4-week follow-up.

All 21 pregnant women with malaria were anaemic (10 ± 1.5 g/dl) before treatment, when they were first screened. Subsequent follow-up after CQ/SP administration within a fortnight revealed that all 6 cases who were treated with SP on day 2, had their Hb level increased by 1 g/dl (2 cases) or remained stable (4 cases). While in women who remained on CQ, their anaemia had further worsened between 6 and 8 g/dl.

Though this study revealed CQ resistant *P. falciparum* in all pregnant women, it clears clinical symptoms rapidly. Further, treatment with CQ may not improve the anaemia of pregnancy. Studies from Thailand[8] reported that incidence of anaemia was proportional to the number of parasitaemic episodes of malaria. Regarding level of resistance, the RI, RII and RIII classification may have its limitation in pregnant women because of the parasite sequestration in the placenta. Further, the number of women was too small to draw definitive conclusion whether CQ resistance/anaemia was more prevalent in primigravidae. However, the overall very high percentage of early failure (RIII) and frequent recrudescence indicate that CQ is no longer useful for the treatment of uncomplicated falciparum malaria in

pregnancy in this area, necessitating strategies to cope with resistant cases. This also raises the question of chemoprophylaxis. The proportion of patients failing to be treated successfully with chloroquine, one of the largest reported from MP to date, may force major policy reviews in the near future. Therefore, it is recommended that the second line antimalarial SP may be tried as done in Malawi, Africa[9].

1. WHO, Report of an informal consultation, Document WHO/MAL/94, 1070, Geneva, 14–18 March 1994.
2. Singh, N., Saxena, A., Chand, S. K., Valecha, N. and Sharma, V. P., *Southeast Asian J. Trop. Med. Public Health*, 1998, **29**, 1–8.
3. Singh, N., Shukla, M. M. and Sharma, V. P., *Bull. WHO*, 1999, **77**, 567–571.
4. Lilijveld, J. and Kortmann, H., *Bull. WHO*, 1970, **42**, 477–479.
5. WHO, Report, Document WHO/MAL/96, 1077, Geneva.
6. Rieckmann, K. H., *Bull. WHO*, 1990, **68**, 759–760.
7. Sahli, H., Lehrbuch der klinischen unter Suchungs methoden, Leip zig: Deuticke, 1894.
8. Nosten, F., Kuile, Ter, F., Maelankin, L., Decludt, B. and White, N. J., *Trans. R. Soc. Trop. Med. Hyg.*, 1991, **85**, 424–429.
9. Schultz, J. L., Steketee, R. W., Chitsulo, L., Macheso, A., Kazembe, P. and Wirima, J. J., *Am. J. Trop. Med. Hyg.*, 1996, **55**, 87–94.

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Understanding the gastric mill structure of a macrophagous shrimp *Metapenaeus monoceros* (Fabricius)

Most of the decapod crustaceans, primarily the shrimps (Penaeids, Palaemonids) are considered as predators or scavengers and are omnivores or detritivores. Their digestive tract has sophisticated and complex mechanisms for trituration and separation of fine food particles, using various appendages and calcified parts of the stomach. In fact, most of the chewing action on food particles occurs in the stomach by a set of triple 'teeth-like structures'. The most unique characteristic of their digestive system is that the fore and hind parts are composed of protein–chitin material and as a part of exoskeleton this layer is removed and replaced in each molt. Even the set of hard-chewing parts located in the stomach are lost and rebuilt at each molt.

The preliminary description of the digestive tract in Crustacea was originated in the 18th century[1]. But the monumental work by Mocquard[2] was first to illustrate the foregut of over 100 species of crustaceans. Patwardhan[3] reviewed the efficiency of internal masticatory apparatus and established an inverse correlation of it with mandibles in Decapoda. Maynard and Dando[4] provided a clue for the basic identification of calcareous ossicles supporting the foregut.

Works on shrimps by Patwardhan[3], Young[5], Meiss and Norman[6], and Suthers[7] described and illustrated the 'gastric mill' of few species of *Penaeus* genus. Scanning electron micrographs of 'gastric mill' from *Penaeus setiferus* are found from the work of Felgenhauer and Abele[8]. But as a generalized comparative work, the details of surface topography of this area were lacking. The gastric mill structure of a shrimp of *Metapenaeus* genus, i.e. *Metapenaeus monoceros*, its surface topography and probable function are described here.

The foregut of freshly collected shrimps (*M. monoceros*) from Hooghly–Matlah estuary was dissected by micro-needle for viewing the internal surface. Double fixation in 4% gluteraldehyde and 2% OsO_4 solution followed by CPD and gold–palladium coating was done. Scanning electron micrographs were taken at 18–20 kV accelerating voltages by JEOL JSM-5200 microscope at USIC, Jadavpur University.

In *M. monoceros*, a total of 17 ossicles were found. The single plate-like urocardiac ossicle occurs on the dorsal median region of the posterior cardiac stomach. Posteriorly, this ossicle curves inward towards the median plane at the floor of the cardiac stomach to form the median tooth (mt). The paired zygocardiac osicles located from outside the foregut form invaginations into the cavity of the lateral cardiac sac along the anteroposterior axis, as a pair of opposing lateral teeth (lt). It is the presumed action of the urocardiac and zygocardiac ossicles and their respective internal sclerotized teeth that forms the 'gastric mill' (Figure 1 *a*).

Close observation at the level of triangular median tooth shows that its margins extend outward from its anterior tip. Each lateral side of the tooth bears closely-packed denticles which increase in size towards the apex. The extreme lateral denticles sharply curve inwards. The surface topography does not show any grinding area (Figure 1 *b*). Paired lateral teeth are dorsally curved and heavily calcified. Paired denticles being reduced in size towards its apex are found. The denticles are slightly curved outwards. Generally 10–12 pairs of denticles are found in fully mature adults (Figure 1 *c*). These numbers are reached with increase in the size of the animal after each molt; the number even increases beyond this limit. The denticle topography clearly shows grinding surfaces (black arrows, Figure 1 *d*). Shrimps are reported to feed on