ORIGINAL ARTICLE

Comparative Study of Effectiveness and Resistance Profile of Chloroquine and Sulfadoxine-Pyrimethamine in Uncomplicated *Plasmodium falciparum* Malaria in Kolkata

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Abstract

Introduction: Malaria is one of the major public health problems of the country. Factors responsible for re-emergence of malaria in India was due to emergence and spread of chloroquine resistant *Plasmodium falciparum* strains across the country coupled with steady rise in insecticide resistance of the vector mosquitoes. Very little is known about the drug resistance status of *P. falciparum* in India. As per National Vector Borne Diseases Control Programme (NVBDCP), chloroquine is the drug of choice for uncomplicated *P. falciparum* cases and the combination of Artesunate and Sulfadoxine-Pyrimethamine (SP) is being used to treat the documented chloroquine-resistant uncomplicated cases. To evaluate the comparative effectiveness and resistance profile of Chloroquine vis-à-vis Sulfadoxine-Pyrimethamine (SP) in uncomplicated *Plasmodium falciparum* cases as the first-line therapy a study was undertaken at the Malaria Clinic of Calcutta School of Tropical Medicine, Kolkata during the period from July 2007 to December 2007 at Kolkata Municipal Corporation, Kolkata.

Material & Methods: Following WHO protocol 2003, a total of 100 parasitologically confirmed *Plasmodium falciparum* cases were recruited as per the recruitment criteria. Among them, 50 patients were given Chloroquine and another 50 patients were given SP. Eight patients were excluded or lost to follow-up during the follow-up period because of failure to follow the protocol.

Results: It was observed that in the Chloroquine group out of 50 patients, 30 (60%) showed adequate clinical and parasitological response (ACPR), 15 (30%) had late treatment failure (LTF) and remaining 5 (10%) were lost during the follow up period (LFU). On the other hand in the SP group out of 50 patients, 46 (92%) showed ACPR and only one (2%) had LTF and 3 patients were LFU. The difference of LTF in Chloroquine and Sulfadoxine-pyrimethamine groups was statistically significant (p value < 0.05). Also there was statistically significant difference of the mean parasite clearance time (PCT) of Chloroquine (82.7 hours) and SP group (61.3 hours).

Conclusions: Chloroquine failure rate was high which was well above the WHO recommended cut off threshold for drug policy change (>10%), Sulfadoxine- Pyrimethamine can be used in place of Chloroquine as the first line drug in uncomplicated *P. falciparum* cases.

Editorial Viewpoint

- High chloroquine resistance is very worriesome.
- Sulfadoxine-pyrimethamine achieves parasite clearance at a faster rate compared to chloroquine.
- Sulfadoxine-pyrimethamine is a very good option for first-line therapy in uncomplicated falciparum malaria where chloroquine resistancs is rampant.

Introduction

Malaria is the most important of the parasitic diseases of humans, with transmission in 107 countries containing 3 billion people, infecting approximately 5% of the world's population and causing 1-3 million deaths

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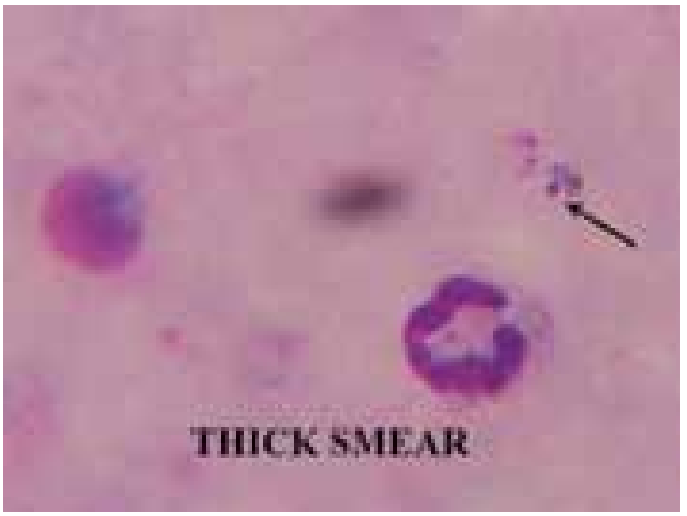

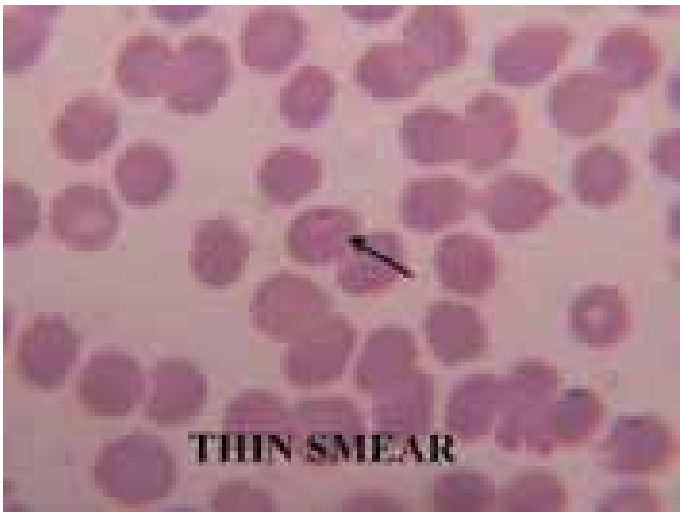

Figs. 1 and 2 : Thick & thin blood smear showing Giemsa stained *Plasmodium falciparum* ring under oil immersion with 100X magnification

each year. Malaria is one of the major public health problems of the country. Around 1.5 million laboratory confirmed cases of malaria are reported in the country annually. Out of the total malaria cases, 40-50% is *Plasmodium falciparum*. About 1.785 million cases of malaria (including 0.839 million *P. falciparum* cases) and 1708 deaths were reported from the country in 2006. Recently 1.525 million cases of malaria (including 0.756 million *Plasmodium falciparum* cases) and 935 deaths were reported in 2008 (Provisional data given by National Vector-Borne Diseases Control Programme (NVBDCP) in Feb 2009).[1]

Major factors responsible for reemergence of malaria in India was due to emergence and spread of chloroquine-resistant *Plasmodium falciparum* strains across the country coupled with steady rise in insecticide resistance of the vector mosquitoes. The first evidence of chloroquine (CQ) resistant *P. falciparum* was noted in India in 1973 from Diphu area of Karbi Anglong district of Assam. Several reports of resistance were subsequently confirmed from Arunachal Pradesh, Andhra Pradesh, Assam, Chhattisgarh, Goa, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Meghalaya, Mizoram, Nagaland, Orissa, Rajasthan, Tripura, Uttar Pradesh, Karnataka, West Bengal, and Andaman Nicobar Islands. In West Bengal, during 1982-1988, Pandeya et al.[2] reported one R-III focus in Purulia and two R-III foci in Jalpaiguri. In recent years multidrug-resistant *P. falciparum* malaria has also been reported from various countries. Very little is known about the drug resistance status of *P. falciparum* in India. As per NVBDCP, chloroquine is the drug of choice for uncomplicated *P. falciparum* cases and the combination of Artesunate and Sulfadoxine-Pyrimethamine is being used to treat the documented Chloroquine-resistant uncomplicated cases. There has been a steady rise in the proportion of recurrent *P. falciparum* cases (following use of recommended doses of chloroquine) attending the Malaria Clinic of School of Tropical Medicine, Kolkata (32.7% in 2001 and 66.5% in 2005). A significant proportion of recurrent *P. falciparum* cases are due to chloroquine resistance.[3] With continued use of chloroquine as first-line of therapy, the number of people with RI resistance and consequently the parasite burden in the community are also increasing. This results in increased transmission of *P. falciparum* in the community. Recently deaths due to *P. falciparum* malaria have been recorded in Kolkata, foothills of Purulia and certain Tea Estates of Dooars area of Jalpaiguri (Government data). While Chloroquine remains the first-line drug for uncomplicated *P. falciparum* malaria as per NVBDCP, a section of doctors, in private as well as in government sector, are using the Artemisinin derivatives and Quinine indiscriminately to treat uncomplicated *P. falciparum* malaria cases. Injudicious use of such reserved antimalarial drugs might lead to development of rapid resistance against them. In endemic areas it's difficult to differentiate "recrudescence" and "re-infection" clinically and/or parasitologically.

This prompted us to undertake a pilot study to evaluate the effectiveness and resistance profile of Chloroquine vis-à-vis Sulfadoxine-Pyrimethamine in uncomplicated *Plasmodium falciparum* cases as the first-line therapy.

Patients and Methods

Study sites: The present study was undertaken at the Malaria Clinic of Calcutta School of Tropical Medicine, Kolkata during the period from July 2007 to December 2007 under Ward no. 44 of Kolkata Municipal Corporation, Kolkata.

Table 1 : Dosage schedule of Chloroquine + Primaquine

Age in years	Day 1			Day 2	Day 3
	Chloroquine tablet	Primaquine		Chloroquine tablet	Chloroquine tablet
		Base in mg	No. of tablets		
< 1	½	NIL	0	½	¼
1 - 4	1	7.5	1	1	½
5 - 8	2	15	2	2	1
9 -14	3	30	4	3	1½
≥ 15	4	45	6	4	2

Chloroquine tablet: 150 mg base, Primaquine tablet: 7.5 mg base

Table 2 : Dosage schedule of Sulfadoxine-Pyrimethamine + Primaquine

Age in years	Sulfadoxine + Pyrimethamine (on Day 1)			Primaquine (on Day 1)	
	Sulfadoxine (mg base)	Pyrimethamine (mg base)	No. of tablets	Base in mg	No. of tablets
< 1	125	6.25	¼	NIL	0
1 - 4	500	25	1	7.5	1
5 - 8	750	37.5	1½	15	2
9 – 14	1000	50	2	30	4
≥ 15	1500	75	3	45	6

Sulfadoxine + Pyrimethamine tablet: 500 mg SDP + 25 mg Pyrimethamine, Primaquine tablet: 7.5 mg base

Patients: A total of 100 patients were enrolled in this study (50 patients each in Chloroquine and Sulfadoxine-Pyrimethamine group). The patients of confirmed *Plasmodium falciparum* malaria from Malaria Clinic were randomly screened and finally recruited for the study using following inclusion and exclusion criteria.

Inclusion criteria

a. Patients belonging to ward no. 44, Kolkata Municipal Corporation
b. Of either sex and above the age of six months
c. Microscopically proved cases of *Plasmodium falciparum* (monoinfection)
d. Parasite density between 1,000-100,000/μL of blood
e. Axillary temperature of ≥ 37.5 ^{0}C or history of fever in previous 24 hours
f. Ability to follow up visits and easy access to health facilities
g. Informed consent of the patient/ parent/guardian

Exclusion criteria

a. History of taking antimalarials within 15 days preceding the illness
b. Presence of mixed infection
c. Inability to provide informed consent
d. Appearance of any of the criteria of severe or complicated malaria during the present illness
e. Pregnancy and history of amenorrhoea
f. Patients with sulfonamide hypersensitivity and known G6PD deficiency

Treatment & follow up: Study design was done as per WHO protocol 2003. This protocol consists of recording essential patient information, clinical assessment, axillary temperature, parasitemia, bodyweight on day 0 (prior to treatment) and with the stipulated drug, clinical assessment with examination of axillary temperature on Days 1,2,3,7,14,21 & 28 and parasitological examination on Days 2,3,7,14,21 & 28. On Day 1 or any other day the patient was also examined for parasitemia, if he/ she had any danger sign or clinical deterioration. Both thick and thin smears were taken in the same slide from finger pricked blood sample, were stained with Giemsa stain and examined under oil immersion lens microscope on Day 0, 2, 3, 7, 14, 21, 28 and on any unscheduled day.

Parasite load was measured by counting number of asexual forms of *Plasmodium falciparum* parasites against 200 leucocytes present in stained thick blood smear. In case of low parasitemia (less than 10/200 leucocytes), counting was done against 500 leucocytes. The parasite load was calculated by applying the following formula.

Parasitaemia (per micro-litre) = Number of parasites × 8000/ Number of leukocytes

As per National Drug Policy on Malaria (2008) by NVBDCP the dosage schedule was as shown in Tables 1 and 2.

Classification of Therapeutic Response According to WHO Protocol 2003

There are three categories of therapeutic responses,[23,24] namely 'Early Treatment Failure' (ETF), 'Late Treatment Failure' (LTF) and 'Adequate Clinical and Parasitological Response (ACPR)'. These are defined as follows:

Early Treatment Failure (**ETF**) if the patient develops one of the four conditions during the first three days of follow up.

- Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitemia
- Parasitemia on Day 2 higher than Day 0 count irrespective of axillary temperature
- Parasitemia on Day 3 with axillary temperature ≥37.5 degree centigrade
- Parasitemia on Day 3 greater than or equal to 25% of the count on Day 0

Late Treatment Failure (**LTF**) is of 2 types:

1. Late Clinical Failure **(LCF)**
   - Development of danger signs or severe malaria after Day 3 in presence of parasitemia, without previously meeting any criteria of Early Treatment Failure

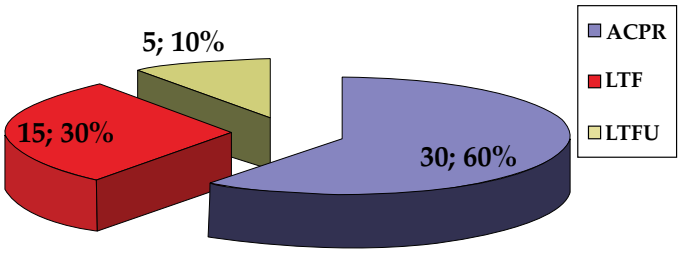

Fig. 3 : Treatment response in chloroquine group
ACPR: Adequate clinical and parasitological response; LTF: Late treatment failure; LTFU: Lost to follow-up

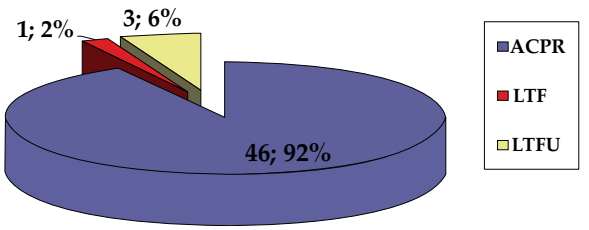

Fig. 4 : Treatment response in sulfadoxine pyrimethamine group
ACPR: Adequate clinical and parasitological response; LTF: Late treatment failure; LTFU: Lost to follow-up

- Presence of parasitaemia and axillary temperature ≥ 37.5°C on any day from Day 4 to Day 28, without previously meeting any of the criteria of Early Treatment Failure

2. Late Parasitological Failure **(LPF)** : Presence of parasitaemia on any day from Day 7 to Day 28 and axillary temperature <37.5°C, without previously meeting any of the criteria of Early Treatment Failure or Late Clinical Failure

Adequate Clinical and Parasitological Response **(ACPR)** : Absence of parasitaemia on Day 28 irrespective of axillary temperature without previously meeting any of the criteria of Early Treatment Failure or Late Clinical Failure or Late Parasitological Failure

Fever Clearance Time **(FCT)** : This is the time from beginning of antimalarial treatment until the patient is apyrexial. This is of two types - FCTa and FCTb. FCTa is when temperature first falls below 37.5°C (99.5°F) and FCTb is when the temperature falls and remains below 37.5°C for 24 hours. In this study FCTa has consistently been taken into consideration.

Parasite Clearance Time **(PCT)**: It is the time between beginning the antimalarial treatment and the first negative blood slide.

Results

In the present study a total of 100 parasitologically confirmed *Plasmodium falciparum* cases belonging to ward no 44 under Kolkata Municipal Corporation were recruited during the period from July 2007 to December 2007 as per the recruitment criteria. Among them, 50 patients were given Chloroquine and another 50 patients were given Sulfadoxine-Pyrimethamine. Eight patients were excluded or lost to follow-up (five patients in the Chloroquine arm and 3 patients in the Sulfadoxine-Pyrimethamine arm) during the follow-up period because of failure to follow the protocol (antimalarial treatment administered by themselves or a third party) or failure to come for follow-up on the scheduled days.

There were 87 male patients (87%) and 13 female patients (13%). In the Chloroquine arm there were 42(84%) male patients and 8 (16%) female patients and in the Sulfadoxine-Pyrimethamine arm there were 45 male patients (90%) and 5 (10%) female patients.

The mean age at presentation was 32.4 years (Range 10-62, Median 30). For Chloroquine arm the mean age was 33.3 years (Range 10-62, Median 32.5) and for Sulfadoxine-Pyrimethamine arm the mean age was 31.5 years (Range 13-62, Median 29.5).

According to the study protocol, *Plasmodium falciparum* parasitemic patients with fever of >37.5°C (>99.5°F) or with history of fever within the previous 24 hrs were enrolled. On Day 0, the mean temperature in the Chloroquine group was 100.56°F (range 96-103.6°F) and that of Sulfadoxine-Pyrimethamine group was 100.63 ^{0}F (range 96.8-103.8°F). Six patients were afebrile at day 0; 4 in the Chloroquine group and 2 in the Sulfadoxine-Pyrimethamine group. All of them had a history of fever within the previous 24 hours.

The mean Fever Clearance Time (FCT) of 93 patients was 29.9 hours (range 24-72 hours) and that of Chloroquine and Sulfadoxine-Pyrimethamine arm was 28.8±13.145 hours (median 24 hours; range 24-72 hours), and 31±14.818 hours (median 24 hours; range 24-72 hours) respectively. The difference of mean FCT of Chloroquine and Sulfadoxine-Pyrimethamine group was not statistically significant (p = 0.45).

On day 0, the mean parasite count of Chloroquine group was 10409.6 per µL (range 1040-95840 /µL) and that of Sulfadoxine-Pyrimethamine group was 9984.04 /µL (range 1040-72000/µL)

The mean Parasite Clearance Time (PCT) of 94 patients was 72 hours (range 48-168 hours) and that of Chloroquine and Sulfadoxine-pyrimethamine arm was 82.7 ± 40.287 hours (range 48-168 hours) and 61.3 ± 25.423 hours (range 48-168 hours), respectively. The difference of mean PCT of chloroquine and Sulfadoxine-Pyrimethamine group was statistically significant (p value = 0.003).

In the chloroquine arm, out of the 50 patients, total 30 patients (60%) showed Adequate Clinical and Parasitological Response (ACPR), 15 patients (30%) showed Late

Treatment Failure (LTF) [among them 9 patients had actually Late Clinical Failure (LCF i.e. fever > 99.5°F in presence of parasitaemia) and 6 had Late Parasitological Failure (LPF i.e. parasitaemia without fever)] and 5 patients (10%) were lost during follow up period.

In the Sulfadoxine - Pyrimethamine arm, out of the 50 patients, total 46 patients (92%) showed Adequate Clinical and Parasitological Response (ACPR); only one patient (2 %) showed Late Treatment Failure (LTF)[actually Late Parasitological Failure (LPF)] and 3 (6%) patients were lost during follow up period.

This difference of LTF was significant (p value < 0.05).

Discussion

Chloroquine resistance status

In our study, it was observed that in the Chloroquine group out of 50 patients, 30 (60%) showed Adequate Clinical and Parasitological Response (ACPR), 15 (30%) had Late Treatment Failure (LTF) and remaining 5 (10%) were lost during the follow-up period.

Similar studies with Chloroquine conducted in different parts of the world showed varied results. Those are described below:

In 2000 Kulkarni et al[4] reported 62.5% LTF cases in Mumbai. Ghosh et al[5] in Madras showed 5 resistance cases out of 6 samples and in Jabalpur, Madhya Pradesh 12 (85.7%) resistance cases out of 14 samples. Das Khatri[6] in 1991 reported 24% ACPR (out of 98 cases), 54% R I, 4% R II and 9% R III in Rajasthan. In West Bengal, Pandeya et al[2] showed three R III foci - two in Jalpaiguri and one in Purulia. The studies showed a parasite clearance of 40% and 32% within seventh day in Purulia and Jalpaiguri districts, respectively. Biswas S [3] (2005) reported 30% ACR, 54% LFT & 16% ETF in chloroquine treated cases from School of Tropical medicine, Kolkata. Recently, a study has been carried out from School of Tropical Medicine, Kolkata by Maji et al[7] (unpublished data) on status of antimalarial drug resistance of *Plasmodium falciparum* malaria in Uttar Latabari BPHC, Kalchini block & Dhumpara PHC, Nagrakata block in the district of Jalpaiguri in 2007-08. In Kalchini Block, the treatment failure rate of Chloroquine was 59 % (ETF 11.3% & LTF 47.7% cases) and the ACPR was 41%. In Dhumpara, Nagrakata Block, the treatment failure rate of Chloroquine was 73% (ETF 14.6% & LTF 58.4% cases) and ACPR rate was 27%. In Pakistan, Khan et al[8] in 2004 reported up to 16-62% chloroquine-resistant *Plasmodium falciparum* cases. Rahman et al[9] reported 56% ETF cases from Bangladesh in 1996-97. Maguire et al (2002)[10] in Central Java, Indonesia reported 36 (47 %) treatment failure. Checchi et al[11] in Harper, south-west Liberia reported chloroquine failure rate of 84.0% (95% CI 70.9-92.8%). Moses et al[12] in Kampala, Uganda showed 54% clinical failure and 72% parasitological failure. Fever clearance at day 3 was 85%.

Mahapatra et al[13] in Changlang and Lohit districts of Arunachal Pradesh recorded 23.8% ETF, 14.3% LCF, 10.7% LPF and 51.2% ACPR. Schwobel et al[14] in Lao PDR showed 44.8% early or late treatment failure. Checchi et al,[15] in Sierra Leone, showed chloroquine failure proportions were ranging from 39.5% in Kabala to 78.8% in Kailahun. Early failures under CQ were frequent.

Sulfadoxine-Pyrimethamine resistance status

In the present study, out of 50 patients in the Sulfadoxine-Pyrimethamine (SP) group, 46 (92%) showed Adequate Clinical and Parasitological Response (ACPR) and only one (2%) had Late Treatment Failure (LTF) and 3 patients (6%) were lost during follow up period.

In Pakistan, Khan et al[8] showed four to 25% of cases were resistant to sulfadoxine-pyrimethamine. A recent study in 2007-08 by Maji et al[7] (unpublished data) documented that in Kalchini Block, Jalpaiguri, the treatment failure rate of Sulfadoxine-Pyrimethamine was 19.5% (ETF 13% and LTF 6.5%) and the ACPR was 80.5%. In Nagrakata Block of the same district, the treatment failure rate of Sulfadoxine-Pyrimethamine was 12% (ETF 2% & LTF 10%) and the ACPR was 88%. Bijil et al[16] (2000) reported 26% sulfadoxine-pyrimethamine resistant cases in Africa. Maguire et al[10] (2002) in Central Java, Indonesia reported 22% treatment failures to SP. Almost similar results were observed in a study of SP resistance from Tanzania by Mugittu et al (2005).[17] They had documented 50.9% ACPR at Day 28 and 17.1% and 24.1% of clinical and parasitological failure respectively after 28 days of follow up. In Malawi, Plowe et al[18] (2004) documented treatment failure rate of 20% in SP treated group. Checchi et al[11] in Harper, south-west Liberia reported SP failure rate of 51.5%. In Gambia, Bojang et al[19] showed ETF was 10.68% and LTF was 10% in SP treated patients. In Gambia another study by Muller et al[20] showed ETF & LTF in Sulfadoxine-Pyrimethamine group were 17% and 14% respectively. In Uganda, Talisuna et al[21] observed parasitological failure was 61%. Checchi et al[22] in Western Uganda reported 37% LTF & 15.2% ETF. Moses et al[12] in Kampala, Uganda reported 11% clinical failure and 30% parasitological failure among SP treated group.Mahapatra et al[13] in Changlang and Lohit districts of Arunachal Pradesh recorded 14.1% ETF, 12.6% LCF, 8.1% LPF and 65.2% ACPR. Schwobel et al[14] in Lao PDR documented 17.9% early or late treatment failure. Checchi et al, in Sierra[15] Leone, observed that the SP failure rate varied from 23.2% in Kabala to 46.1% in Kailahun.

From the above discussion it is evident that the Chloroquine failure rate in Ward no. 44 of Kolkata

Municipality Corporation is high (30%) which is well above (>10%) the WHO recommended cut-off threshold for drug policy change, Sulfadoxine-Pyrimethamine can be used in place of chloroquine as the first-line drug in uncomplicated *P. falciparum* cases either alone or in combination with Chloroquine. Chloroquine failure rate was very much high in Jabalpur (85.7%) and in Harper, Liberia (84%) reported by Ghosh et al[5] and Checchi et al[11] respectively. Although the SP failure rate in other countries was very high but in our study in Kolkata it was only 2%. Whether these high failure rates was due to recrudescence (i.e. true failure/ resistance) or reinfection can only be confirmed by genetic study (PCR or DNA fingerprinting). Only 4 studies (Pandeya et al,[2] De et al, Biswas et al[3] and Maji et al[7]) were done in West Bengal. Again our study gives an idea about the resistance pattern of central Kolkata only. To know the resistance pattern of whole of Kolkata similar studies should be carried out in other parts of the city viz. southern, north-eastern fringe and northern parts. It's also important to carry out such studies in other malaria endemic districts of West Bengal (e.g. Jalpaiguri, Paschim Midnapur, Bankura and Purulia) for better understanding of the antimalarial drug sensitivity pattern in the state. It will be useful to establish few sentinel sites in malaria endemic districts of West Bengal to monitor the therapeutic efficacy of different anti malarial drugs.

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