STUDY OF CHLOROQUINE RESISTANCE IN *Plasmodium falciparum* INFECTION IN NORTH EAST INDIA

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ABSTRACT

During the period April 1993 to July 1993, a total of 410 patients with pyrexia were screened for malarial parasite and 109 (26.5%) cases were found positive for malarial parasite. Of these 109 cases, 91 were positive for *Plasmodium falciparum*. Of these 91 cases, 43 patients fulfilled the study criteria as per WHO's extended field trial and were included in the analysis. These patients were treated with standard doses of chloroquine. Seven patients each (16.3%) showed RI and RII resistance and 2 patients (4.7%) showed RIII levels of chloroquine resistance.

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KEYWORDS : Chloroquine; Drug resistance; *Plasmodium falciparum*; Malaria.

Introduction

Drug resistance to chloroquine was first reported in 1973 from Assam [1]. Since then it has been reported from all parts of India [2-5]. In many parts of the country the resistance is of a high degree, requiring frequent use of quinine and terramycin combination as a life saving measure. Therefore there is a need to monitor chloroquine resistance and know the exact degree of resistance periodically. There are many studies to this effect [2-5] but there is paucity of studies from service hospitals [2,6]. Since the soldiers are deployed in malarious areas in north-eastern sector we undertook this study to find out the present status of chloroquine resistance.

Material and Methods

All cases of fever admitted to a service hospital in Assam from April 1993 to July 1993 were screened for malarial parasite (MP) by examining thick and thin peripheral smears. Cases found positive for asexual forms of *Plasmodium falciparum* were studied as per WHO's extended field trial [7,8].

After Leishmann staining, slides were analyzed to count parasite per 300 WBC's. Subsequently these counts were converted to count per μ^3 based

on patients WBC count as on admission or later [7].

All patients were admitted and followed-up clinically and by doing parasite count daily for 7 days and then weekly or as necessary for a total of 28 days. The day of start of therapy was taken as Day 1. All the 43 patients studied received chloroquine therapy. Those patients who were stable and without any pernicious complication were given tablet chloroquine sulfate in a dose of 600 mg stat, 300 mg after 6 hours and 300 mg daily for next 2 days. Patients with any pernicious complication were administered quinine and terramycin.

Response to therapy was judged as per WHO's extended field trial criteria as follows :-

S - Clearance of asexual parasitaemia in 7 days and no recrudescence in next 28 days.

RI - Clearance of asexual parasitaemia in 7 days, as above, followed by recrudescence within 28 days.

RII - Marked reduction in parasitaemia (by 75% or more) within 48 h but no clearance by Day 7.

RIII - No marked reduction in parasitaemia (less than 75%) or an increase in parasitaemia within 48 hours.

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All cases of mixed infection and those patients who consumed unquantifiable doses of antimalarials or drugs other than antimalarials were excluded from the study.

Results

A total number of 410 cases with fever were screened during April 1993 to July 1993. Out of a total of 109 patients with slide positive malaria, 91 (83.4%) cases had *P. falciparum* infection, 17 (15.5%) had *P. vivax* infection and 1 patient had mixed infection. Age of these patients ranged between 17 and 53 years with a mean of 23.2 years. Out of 91 cases of *P. falciparum* infection only 43 could be studied. Remaining 48 cases which were studied included 17 females, and others due to incomplete follow-up, and male patients requiring quinine and terramycin from the start of therapy.

Sensitivity status of all the patients is summarized in Table 1. While 37.3 per cent cases showed varying degrees of chloroquine resistance 62.7 per cent were sensitive. RII and RIII patients required quinine and terramycin combination for complete eradication of malarial parasite. On the other hand all RI cases were retreated with chloroquine with good clinical response. However, out of these 7 patients 3 had recrudescence twice in 28 days period. Hence they were also treated with quinine and terramycin combination.

TABLE 1
Drug sensitivity status in 43 patients with *P. falciparum* infection

Sensitivity status	No of patients	Percentage
Sensitive	27	62.7%
RI resistance	7	16.3%
RII resistance	7	16.3%
RIII resistance	2	4.7%

Discussion

Chloroquine resistance is defined as "ability of the parasite to survive or grow despite administration and absorption of a drug given in doses equal to or higher than the usually recommended but within the tolerance of the patient" [8]. In defining drug resistance in malaria, reinfection within a period of 28 days has to be excluded. We achieved

this by strict use of mosquito nets and other anti-malarial precautions during the therapy period.

This study has shown a higher degree of chloroquine resistance than previous studies [2,3,9]. Sehgal [1] Gopinathan [2] and Barkakaty [9] did not find a single case of RIII resistance in north-east India. Barkakaty found RI resistance in 8 out of 23 cases in Sonapur, 16 out of 18 cases in Galeki and 5 out of 14 cases in Baghmara areas of Assam and Meghalaya [9]. The Nagaon and Tejpur districts of Assam, where this study was performed, have been found to have RII level of chloroquine resistance earlier [10] whereas this study has shown 2 (4.7%) patients with RIII resistance. Increase in degree of resistance is probably due to natural selection of resistant strains and regular use of chloroquine as prophylaxis ('Drug pressure') [3,11]. Walliker has proposed how drug pressure operates [12]. Chloroquine resistance has a genetic basis. Widespread use of the drug in low doses (e.g. for prophylaxis and indiscriminate treatment) results in selection of resistant strains. High rate of mosquito transmission of mixed (sensitive and resistant) parasites could bring more than one resistant genes together in a single parasite and result in higher degree of resistance. Reverse of this happens when drug pressure is relieved [12].

Chloroquine resistance arises as a result of a mutation which is clinically stable [8]. As a result of the mutation chloroquine is not able to combine with haemoglobin breakdown products to form toxic metabolites to kill the parasite [8]. Chloroquine resistant *P. falciparum* also has reduced accumulation of chloroquine, either due to slower entry or faster expulsion of the drug [13].

Chloroquine resistance is dose dependent. It has been found that in some cases peak chloroquine concentration achieved was only 0.34 µg/mL with a standard dose of 25 mg/kg body weight [8]. This was insufficient for cure and resulted in early recrudescence because resistant *P. falciparum* needs 0.8-1.14 µg/mL of chloroquine for proper response [8]. A repeat course of 30 mg/kg body weight of chloroquine achieved 1.28 µg/mL concentration and cured patients. This dose was, however, not well tolerated by many patients. Similarly it has been observed that 300 mg base of chloroquine weekly over 6 weeks in healthy volunteers

achieved a concentration of 0.6 µg/mL which was insufficient to prevent chloroquine resistant *P. falciparum* infection [4]. Therefore in areas where chloroquine resistance is highly prevalent, MIC for chloroquine is likely to be high and the usual dose of 300 mg per week will not offer significant protection.

RII and RIII levels of resistance necessitate use of quinine and terramycin for complete cure. RI level of resistance can be retreated with chloroquine [13,14] with prompt clearance of parasitaemia but because of recrudescence in short-term follow-up it is recommended that RI cases should also be treated with quinine and terramycin combination. To conclude, there is a high degree of chloroquine resistance in Tejpur and Nagaon District of Assam and there has been an increase in the level of drug resistance since the previous studies.

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