CHLOROQUINE RESISTANCE AND *PLASMODIUM FALCIPARUM* IN PUNJAB, PAKISTAN DURING 2000-2001

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Abstract. During the years 2000-2001, the rural populations of 5 districts in Punjab were examined for malarial parasites. The incidence of *Plasmodium falciparum* was more than double (8.98%) that of *P. vivax* (4.06%). The incidence was higher among male subjects (53.5%) than females (46.9%). The largest number of infected male subjects was found in Sheikhupura district (77.78%). Chloroquine resistance was only checked in the subjects harboring *P. falciparum*, using *in vivo* techniques. Overall chloroquine sensitivity was 63.8%. Overall frequency of chloroquine resistance in the 5 Punjabi districts was 35%, with 30.6% RI and 4.4% RII. It is important that RIII was not found in the present study. Among the five districts, maximum RI (35.1%) and RII (5.4%) were noted in Multan. By age, maximum chloroquine resistance was noted in the 1-5 year age group (*ie* RI, 41%; RII 8%). A similar RI value (41%) was noted for the 6-14 age group, but with a low RII (3%) value. Although, the present finding is an outcome of a survey conducted in only 5 districts of Punjab, it reflects an alarming situation, as not only RI and RII resistance against chloroquine is increasing, but at the same time the incidence of *P. falciparum* is increasing two-fold that of *P. vivax*. The findings warrant that top priority be given to determining the exact status of chloroquine resistance among *P. falciparum* in this region, which is now hosting a heavy influx of refugees from Afghanistan, a country endemic for *P. falciparum*.

INTRODUCTION

The problem of malaria has been exacerbated in recent years by the development and rapid spread of resistance among *Plasmodium falciparum* to the more commonly-used and affordable antimalarial drugs (Bloland, 2001). Resistance among *P.falciparum* is more common, particularly in the malaria-endemic countries (WHO, 2001a,b). Pakistan, one of the 5 countries with moderate endemicity (WHO, 2001b), is also facing this problem (Rana *et al*, 1997, 1998). Chloroquine is gradually losing its efficacy as a single first-line drug treatment following the evolution of resistance.

In Pakistan, each subsequent study has revealed the existence of resistant strains and even worsening degrees of resistance in some cases, where RIII resistance to chloroquine and fansidar have been reported against *P. falciparum* (Yousaf and Nadeem, 1996). In view of this alarming situation, the present study aimed to determine the response of *P. falciparum* to a standard dose of chloroquine in Punjab, Pakistan. This will help predict future trends of drug resistance, appropriate remedies and control measures, including a change in the first-line drug.

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MATERIALS AND METHODS

Study design and sampling technique

The rural populations of Sheikhupura, Muzaffargarh, Multan, Jhang, and DG Khan districts in Punjab Province were selected. A longitudinal non-randomized antimalarial drug resistance trial with *in vivo* technique was designed (WHO, 1973). A multistage cluster sampling technique was used to identify the study subjects. Selection of area was based upon: i) high endemicity of malaria measure by Annual Parasite Incidence (API) and/or ii) high proportion of *Plasmodium falciparum* compared to *P.vivax*.

In each test site established, at least 30 subjects who met the eligibility criteria were selected

for recruitment into the intervention trial. The inclusion criteria were: i) single species infection; ii) a minimum threshold of 1,000 sexual parasites but <80,000 asexual parasites per cubic millimeter of blood; iii) the patient had not received any of the following anti-malarial drugs for the specified period: 4 aminoquinolines (chloroquine) (14 days), sulfadoxine-pyrimethamine (fansidar) (4 weeks), mefloquine (6 weeks).

Different data collection tools, containing structured questionnaire for the identified variables had been developed, pretested, revised, and used.

Study period

Following the *in-vivo* techniques recommended by the WHO (1973), a chloroquine resistance study was conducted in the randomly selected areas in seasons with low transmission to minimize the risk of reinfection.

Quality assurance

Urine was examined on days 0 and 2 for absorption of chloroquine by Dill-Glazko test and sulfonamides by single-paper test (Ashraf, 1993).

Follow-up of test cases

During the follow-up stage, blood slides of all subjects recruited were obtained on days 1,2,3,7,14,21,28 for monitoring the course of asexual parasitemia (Rowland *et al*, 1997).

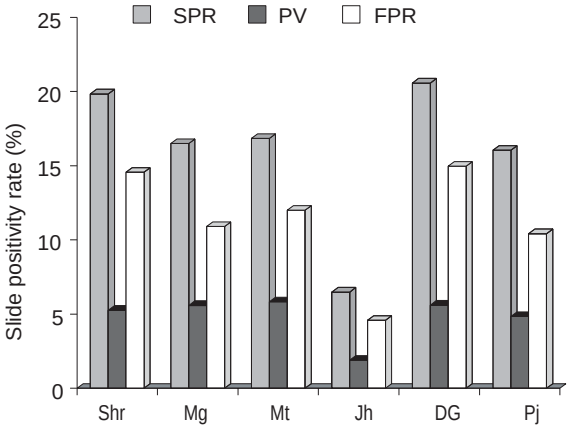
Treatment of resistant cases

Once the resistance of RII and RIII was established, patients were withdrawn from the study and treated with alternative anti-malarial drugs.

RESULTS

In the 5 districts of Punjab studied, the overall incidence of malaria was 13.11%. The results showed that the incidence of *P. vivax* was 4.08% and *P. falciparum* 9.03% (Table 1). Among the 5 districts, maximum *P. falciparum* incidence (14.97 and 14.57%) was found in DG Khan and Sheikhupura, and minimum incidence in Jhang (4.59%) (Fig 1).

The incidence of malaria infection was higher among males (53.5%) than females (46.94%). The maximum for male subjects (77.78) was recorded from Sheikhupura district (Fig 2).



Shr=Sheikhupura; Mg=Muzaffargarh; Mt=Multan; Jh=Jhang; DG=D.G. Khan; Pj=Punjab

Fig 1–Slide positivity rate for *Plasmodium falciparum* and *P. vivax* in different districts of Punjab, 2000-2001.

Table 1
Slide positivity rate for *Plasmodium falciparum* and *P. vivax* in different districts of Punjab, 2000-2001.

Area	Slides examined	PV	PF	SPR (%)	PVPR (%)	FPR (%)
Sheikhupura	247	13	36	19.84	5.26	14.57
Muzaffargarh	1,090	61	119	16.50	5.59	10.91
Multan	925	54	111	16.84	5.83	12
Jhang	1,893	36	87	6.49	1.90	4.59
DG Khan	374	21	56	20.58	5.61	14.97
Total	4,529	185 (4.08%)	409 (9.03%)	16.05	4.84	10.43

PV = *Plasmodium vivax;* PF = *falciparum;* SPR = Slide positivity rate; FPR = Falciparum positivity rate.

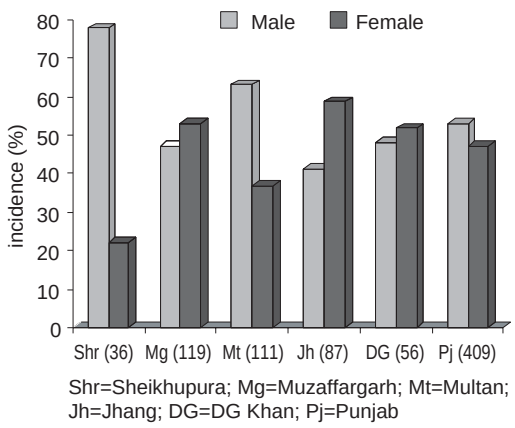


Fig 2–Malaria incidence in male and female subjects from different districts of Punjab, 2000-2001.

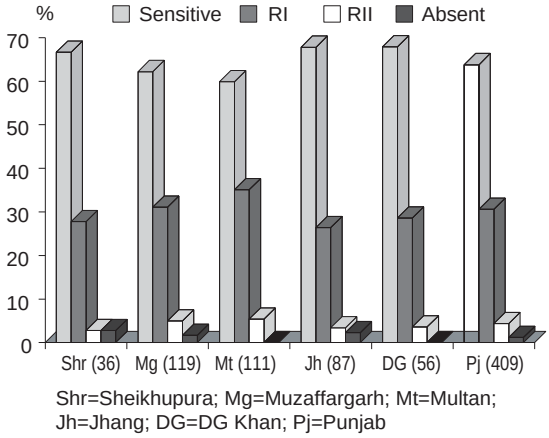


Fig 4–Frequency of chloroquine resistance (%) in different districts of Punjab.

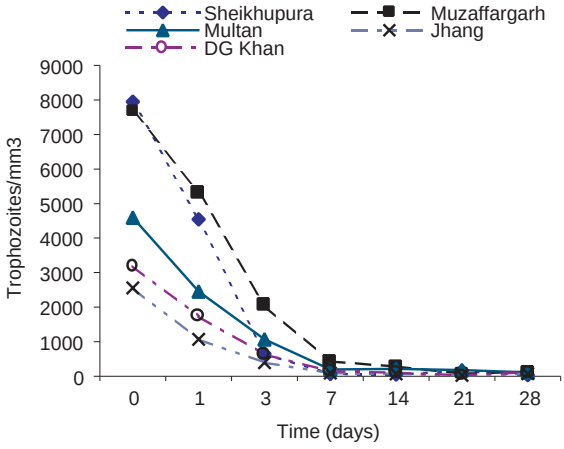


Fig 3–Daily clearance of parasitemia in different districts of Punjab after standard dose of chloroquine.

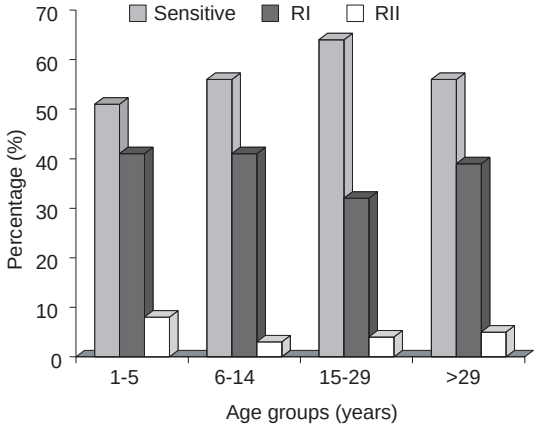


Fig 5–Frequency of resistance (%age) within different age groups in Punjab after standard dose of chloroquine.

The survey showed that 63.8% of patients harboring *P. falciparum* were sensitive for chloroquine (Fig 3), while 35.0% showed resistance (Fig 4), with 30.6 and 4.4% RI and RII values, respectively. In all age groups, the RI and RI values ranged from 39-41%, and 3-8%, respectively (Fig 5).

DISCUSSION

Among the 5 districts, the maximum RI (35.1%) and RII (5.4%) were in Multan district. In this regard, regular surveys have never been conducted, however, the available reports showed that in Punjab, in districts Multan, Muzaffargarh and Jhang, RI and RII against chloroquine was 38.33 and 1.67% (Rana *et al*, 1997). The response of *P. falciparum* infection to chloroquine in Faisalabad district was 54.5% (RI) and 15.1% (RII) (Shah *et al*, 1988).

In Kasur district (Punjab) and in the Afghan refugee camp (NWFP) RI and RII, chloroquine resistance against *P. falciparum* has been reported as 14.8 and 14.28%, and 68 and 2%, respectively

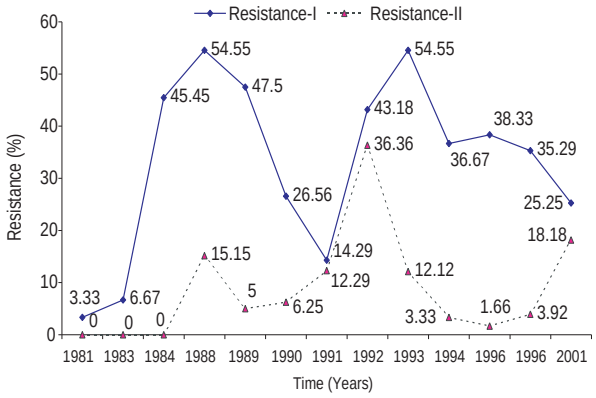


Fig 6–Chloroquine resistance against *Plasmodium falciparum* in Punjab.

(Shah, 1991a,b,c). Until now, no RIII case has been reported from Punjab. In the present investigation, the 1-5 year age group showed maximum RI and RII resistance. However, a similar RI value (41%) was noted in the 6-14 years age group, but with low RII (3%).

Although the present results are an outcome of a survey conducted in only 5 districts of Punjab, they are a cause for serious concern, because not only RI and RII are present, but the incidence of *P. falciparum* is also increasing compared with *P. vivax*. In view of this situation, more organized and thorough studies must be conducted to elucidate the epidemiology, geographic distribution, and degree of drug resistance, to assess the need for changing first-line drug therapy. Previous reports (Fig 6), showed that not only RI and RII against chloroquine are constantly present (with some fluctuations), but the incidence of *P. falciparum* is also increasing. Among the various reasons is that Pakistan is hosting a heavy influx of refugees from Afghanistan, a country endemic for *P. falciparum* (WHO, 2001a).

ACKNOWLEDGEMENTS

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