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RESISTANCE OF PLASMODIUM FALCIPARUM TO CHLOROQUINE IN SOUTH EASTERN IRAN

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ABSTRACT

In vivo and *in vitro* assessments of the response of *P. falciparum* to chloroquine using WHO standard kits and techniques were carried out in Iran Shahr, Sistan and Baluchestan province of Iran in 1985.

In the *in vivo* assessment, 24 malaria patients treated with chloroquine (25mg/kg over three days) were followed up for one to four weeks. The mean parasite clearance time was 4.3 days and in two cases, recrudescence occurred on the 20th and 22nd day.

In the micro *in vitro* test, from among 87 samples, the growth of the parasites was satisfactory in 11 cases (12.6%) and the development of the parasites continued in the presence of higher doses of chloroquine (1.14 to 6.4 micromol/l blood).

In the macro *in vitro* test, from 28 successful tests, the growth of the parasites continued in the presence of higher doses of chloroquine (1.25 to 3 micromol/l blood) in eight cases (28.5%).

The present study showed resistance of *P. falciparum* to chloroquine in Iran Shahr area, southeastern Iran, and confirmed the results of the preliminary studies carried out in this area in 1983.

INTRODUCTION

Malaria is still prevalent in the southeastern parts of Iran. Antimalarial drugs, particulary chloroquine, are used large scale in these areas. The *in vivo* assessment of the response of *P. falciparum* to chloroquine in some malarious areas in southern Iran did not reveal any significant resistance between 1967 and 1976.[1,2] The preliminary *in vivo* and *in vitro* studies (using locally made micro and marco *in vitro* susceptibility test kits) of the response of *p. falciparum* to chloroquine in the Iran Shahr area, Sistan and Baluchestan province of Iran, showed evidence of resistance in 1983.[3]

Further *in vivo* and *in vitro* studies (using WHO standard kits) seemed to be necessary for confirmation of such resistance in Iran Shahr area, where there is considerable movement of population, mainly between the Baluch tribes of Iran and Pakistan and also Afghan immigrants.

MATERIAL AND METHODS

Study area

The study was carried out with the cooperation of the Malaria Eradication and Communicable Diseases Control in the Iran Shahr area in Sistan and Baluchestan province from August 24 to November 16, 1985. The area is located approximately at 26° to 28° North and 60° to 62° East in southeast Iran at an altitude of 566 meters above sea level.

The annual rainfall is about 104mm and the mean annual temperature and relative humidity ranged from 13.4° to 37.7° C (absolute temperature: 1° to 50.5° C) and 4.7 to 73.0% (absolute relative humidity: 2 to 98%), respectively, in 1985.

The population of Iran Shahr area was approximately 246,000 in 1985. Vivax and falciparum malaria are prevalent in the study area with a ratio of 2:5 and total incidence of approximately 20 per 1000 population in 1985.

Selection of cases and testing

Falciparum malaria patients were selected from among out-patients who referred to the Malaria Eradication Laboratory in Iran Shahr. The age of the subjects ranged from one to 60 years and the majority (73.6%) were male.

For each patient, whenever possible, one, two or three *in vivo* and macro *in vitro* chloroquine susceptibility tests were performed. In the *in vivo* assessment, the WHO standard seven day field test[1] was carried out. In some cases, the treated patients were followed up from the second up to the end of the fourth week. The micro and macro *in vitro* tests were perfomed on the basis of the techniques of Reickmann, et al. and Reickmann and Lopez Antunano.[6,7] The *in vitro* susceptibility kits were supplied by the World Health Organization and applied according to WHO guidelines.

RESULTS

In the *in vivo* assessment, from among 29 falciparum malaria patients treated with the standard dose of chloroquine (25 mg/kg over three days), 24 patients 10 to 51 years of age were followed up daily for at least seven days. The asexual forms of *P. falciparum* against 2000 leukocytes in the microscopial examination of the Giemsa-stained thick blood smear had nearly disappeared from day two to day seven. In two cases, a few (one or two) asexual forms were observed on the seventh day. The mean parasite clearance time was 4.3 days.

From six patients who were followed up more than seven days (up to the fourth week) parasitemia, most probably recrudescence, reappeared in two cases on the 20th and 22nd day. One of these was one of the two cases who showed a few asexual froms of the parasite against 2000 leukocytes on the seventh day. The *in vivo* assessment showed resistance of the parasite to the drug at least at the R_1 level.

In the micro *in vitro* test from 148 *P. falciparum* infected blood samples collected from patients 1 to 60 years old, the growth and development of the young trophozoites into schizonts was satisfactory (greater than or equal to 10% in the control vials) in 87 (58.7%) of the samples.

In 11 (12.6%) of these positive cases, the growth of the parasites continued in the presence of higher doses of chloroquine (1.14 to 6.4 micromol/l blood), which indicated *in vitro* resistance of *P. falciparum* to the drug.

In the macro *in vitro* test from 31 *P. falciparum* infected blood samples collected from patients aged 15 to 50 years, the young trophozoites developed into schizonts at least in the control vials in 28 samples (90.3%). In eight (28.5%) of these cases, the growth of the parasites continued in the presence of higher doses of chloroquine (1.25 to 3 micromol/l blood), which again indicated resistance.

In 15 cases in which the growth of the parasites occurred in both micro and marco *in vitro* tests, the response of the strains of *P. falciparum* to chloroquine were coincident; four cases were resistant and 11 cases were sensitive in both tests.

The regression lines of the response of *P. falciparum* to chloroquine in the micro and macro *in vitro* test are shown in Figures 1 and 2.

DISCUSSION

In recent years, chloroquine-resistant strains of *P. falciparum* in Southeast Asia have continued to spread westward and have been officially reported up to the southeastern border of Iran.[9]

Generally, the *in vivo* and *in vitro* susceptibility test data obtained in this present investigation demonstrated resistance of *P. falciparum* to chloroquine in the Iran Shahr area (at least at the R_1 level) and confirmed the results of preliminary studies carried out with locally-made micro and macro *in vitro* test kits in 1983 in the same area.[2]

In the *in vivo* assessment of the response of *P. falciparum* to chloroquine, the mean parasite clearance time was 2.1 days in 1983 and 4.3 days in 1985.

In the *in vitro* tests, the development of young trophozoites of *P. falciparum* into schizonts in the presence of higher doses of chloroquine occurred in up to 3.2 and 6.4 micromol/l blood in the micro test and 1.5 and 3.0 micromol/l blood in the macrotest in 1983 and 1985, respectively.

These data show an obvious increase in the tolerance

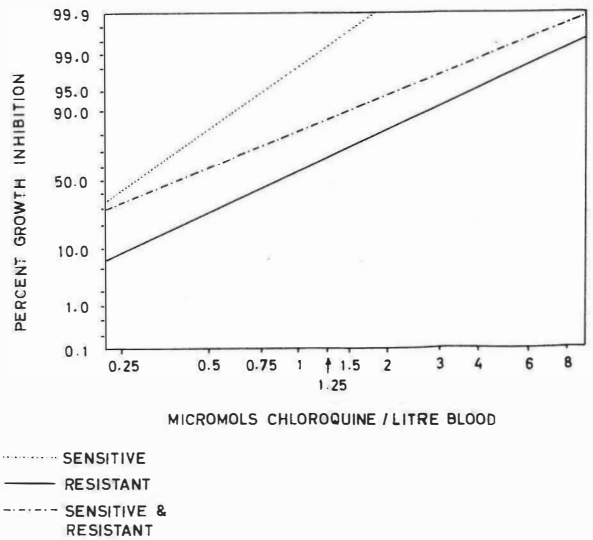

Figure 1. *In vitro* response of *P. falciparum* to chloroquine by the microtest in Iran Shahr, Sistan and Baluchestan province, Iran, 1985.

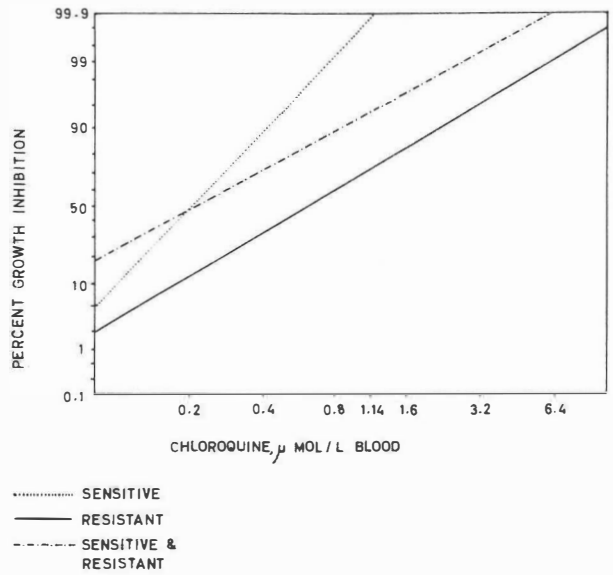

Figure 2. *In vitro* response of *P. falciparum* to chloroquine by the macrotest in Iran Shahr, Sistan and Baluchestan province, Iran, 1985.

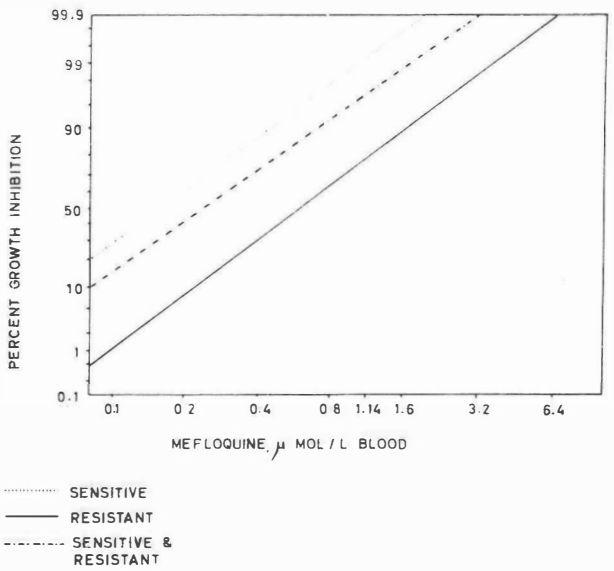

Figure 3. *In vitro* response of *P. falciparum* to mefloquine by the microtest in Iran Shahr, Sistan and Baluchestan Province, 1985.

or the level of resistance of *P. falciparum* to chloroquine in the Iran Shahr area from 1983 to 1985.

The response of *P. falciparum* to mefloquine was also assessed in this investigation. The detailed results of this study have been reported.[4] Relative innate tolerance or resistance to mefloquine was observed in the micro *in vitro* test among strains of *P. falciparum* in the Iran Shahr area, where this drug has never been used. The regression line of the response of *P. falciparum* to mefloquine is shown in Figure 3

The effective concentration of chloroquine and mefloquine required for inhibition of the growth of 50% of the parasites (EC 50) in the present study were 0.228 and 0.229 micromol/l, respectively.[10]

Mefloquine-resistant strains of *P. falciparum* were highly sensitive to chloroquine in the micro *in vitro* test.

Some of the chloroquine-resistant cases were observed among Afghan or Pakistanti individuals or Iranian Baluch tribes who had travelled to Pakistan. Several imported cases of choroquine-resistant falciparum malaria have been also reported among Iranian men returning from India, Afghan and Bangladesh immigrants, and Pakistani tourists.[3]

Therefore, most probably, such migration of the population has imported resistant strains of *P. falciparum* to Iran, and these strains have become established in the malarious areas of the sotheastern parts of the country

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