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Original Article

Evaluation of Sensitivity of *Plasmodium vivax* to Chloroquine

**Nateghpour M [1], Sayedzadeh SA[2], Edrissian GhH[1], Raeisi A [3], Jahantigh A[1], Motevalli-Haghi A[1], Mohseni Gh[4], Rahimi A [1]*

[1]Dept. of Medical Parasitology and Mycology, School of Public Health and Institute of Public Health Research, Medical Sciences/ University of Tehran, Iran
[2] Iranshahr Public Health Training and Research center, Imam Khomeini Boulevard, Iranshahr, Iran
[3] National Malaria Control Programme, Disease Management Center, 68, Iranshahr Ave., Tehran, Iran
[4] Bandar-Abbas Public Health Training and Research Center, Jomhory Boulevard, Bandar-Abbas, Iran

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Abstract

Background: To monitor the current response of *P. vivax* to chloroquine in South and Southeast Iran.

Methods: The study was undertaken from August 2004 until August 2005 at the Bandar- Abbas, Iranshahr, Nikshahr and Chabahar districts. A total of 195 patients out of 225 parasitologically positive *P. vivax* cases completed the study .The patients were given a standard 3- day regimen of chloroquine and followed–up clinically and parasitologically according to the world Health Organization guideline with some modifications. Results of study were addressed as mean of parasite clearance time (MPCT).

Results: The patients responded to the regimen of chloroquine within 24-120 hours. The MPCTs of *P. vivax* for Bandar- Abbas, Iranshahr, Nikshahr and Chabahar districts were 63.05(±15.37), 56(± 21.7), 70.92 (±6.51) and 58(±14) hours, respectively and for the whole study area (South and South East of Iran) was 63.50(±15.84) hours. The results of the whole studied areas indicate that difference of MPCT between male and female patients is marginally significant (P=0.05).

Conclusion: Although, parasite clearance time for a number of cases occurred within 96 and 120 hours, no *P. vivax* parasites had reappeared in considered patients after day five within 28 days follow- up, reflecting that chloroquine is still an efficacious drug for the treatment of *vivax* malaria in the studied districts. Higher MPCT in Nikshahr district than the other districts indicating this could be an early sign for reduced susceptibility of the parasite to the drug.

Keywords: *Malaria*, Plasmodium vivax, *Chloroquine, Iran*

Introduction

Vivax malaria is one of the most important vector-borne diseases in the world. Chloroquine, a 4-aminqunioline antimalarial, has been the drug of choice for the treatment of *vivax* malaria for more than 4 decades in many parts of the malarious areas (1). Although chloroquine, in general, rapidly and effectively eliminates blood schizongy of the parasite, some reports from different parts of the world such as Irian Jaga, Brazil and Myanmar reveal chloroquine-resistance in *Plasmodium vivax* (2- 5). The earliest reports came out by Reickmann et al. (6) and Whitby et al. (7) from Papua New Guinea. Gradually some more reports in the field of chloroquine- resistance in P. vivax were put on the mentioned reports from different malarious areas in the world (8-12).

Malaria is an important infectious disease in Iran and *Plasmodium vivax* is the most common cause of malaria there. According to the report of Centre for Disease Management (CDM) in Iran, 12007 cases of *vivax* malaria have been recorded during March 2004-March 2005, that most of the recorded cases came out from Hormozgan and Sistan & Baluchistan Provinces with 1835 and 6537 cases, respectively. The first attempt for evaluation of susceptibility of *P. vivax* to chloroquine in Iran was made by Edrissian and his colleague (13). Such study was followed by more investigators in Hormozgan province (14, 15).

***Corresponding author:** Tel: +98 21 88989130, E-mail: nateghpourm@tums.ac.ir

We tried to monitor the MPCT of *P. viax* treated with chloroquine in South and South-East Iran.

Materials and Methods

Study areas The study was undertaken from Aug 2004 until Aug 2005 at the Bandar- Abbas district in Hormozgan Province and Iranshahr, Nikshahr and Chabahr districts in Sistan & Baluchistan Province. The Weather in Bandar- Abbas and Chabahar is warm and humid with minimum and maximum temperature about 27.5° C and 33° C, respectively (average 30.2° C) in malaria transmission seasons. Iranshahr and Nikshahr are hot and low humid districts with minimum and maximum temperature about 27° C and 35° C, respectively (average 31° C) in malaria transmission seasons. One to two Health Centers as sentinel sites (according to recorded number of *vivax malaria* during last two yr) were selected in each district.

Plasmodium vivax Is predominant species in the studied areas, and the most important vectors are *Anopheles culicifacies, An. stephersi* and *An. fluviatilis.*

Patients A total of 225 patients (68 from Hormozgan and 157 from Sistan & Baluchistan Provinces) according to the following criteria were initially enrolled for the study, but 30(13.3%) of them did not complete the study. The input criteria for sample size estimation were 95% confidence level, SD of 22.5 (according to the previous study in Bandar-Abbas), precision (d) of 3 for MPCT of *P. vivax* and 10% loss rate of follow up.

Inclusion criteria were patients above 6 mo of age, malaria positive *P. vivax* monoinfection with parasite density of 250-100.000 parasite/ µl, axillary temperature ≥37.5° C or history of fever during the last 24 h, ability to come for the stipulated follow- up visit and easy access to the health facility.

Exclusion criteria were not able to drink or feed, repeated vomiting, convulsions during the present illness, lethargic of unconscious, unable to sit or stand up, presence of a sever disease, presence of sever malnutrition, pregnancy and febrile disease other than malaria.

There were 145 male and 50 female subjects with the age range of 2-67 yr old. Prior to admission a consent form was signed by each of the patients or their guardians.

Study Techniques Bio data and results of initial microscopy examination were recorded in an appropriate form.

The in vivo tests were performed according to the WHO guideline (1) with some modifications. In brief, included patients were treated with a standard regimen of chloroquine (25 mg/kg over 3 d) and in order to achieve a radical cure, primaquine was administered weekly (0.75 mg/ kg) for 8 wk, starting from day 7 owing to avoid interfering in chloroquine efficacy. The in vivo testing was conducted with the purpose of determining the parasite clearance time (PCT). Parasite clearance time was defined as the time from the start of chloroquine treatment until blood films become negative.

Parasite counts were made at day zero and then once on days 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 in the thick blood smears stained with Giemsa. Asexual parasites were counted against at least 200 WBC and then converted to the number of parasites per micro liter of blood.

The results were addressed as a mean of parasite clearance time (MPCT) and analyzed using Microsoft Excel.

Results

The patients responded to the regimen of chloroquine, as shown by the complete clearance of parasitemia, within 24-120 h. No reappearance of *P. vivax* parasitemia was observed in any patients after day 5 at follow-up until day 28. MPCT for the whole study area was 63.50 (±15.84) h with 62.23 (±15.62) and 67.20 (±16.08) h for male and female patients, respectively. The results showed a marginal significant difference between males and females (P= 0.05, df=193 and t=1.92). Details of the results are summarized and illustrated in Table 1. 84.44%, 80%, 70.14% and 45.83% of the patients were male in Bandar Abbas, Iranshahr, Nikshahr and Chabahar districts, respectively. Moreover, the range of age of the studied subjects was 5.5-65, 5-65, 2-55 and 5.5-67 yr old in the mentioned districts, respectively.

Table 1: Parasite Clearance Time of *P. vivax* in response to chloroquine in Bandar- Abbas, Iranshahr, Nikshahr and Chabahar districts, Iran

Province	District		24h. n(%)	48h. n(%)	72h. n(%)	96h. n(%)	120h. n(%)	MPCT(±SD)
Hormozgan	Bandar-Abbas	Male	0	24(47.05)	25(49.01)	2(3.92)	0	61.64(±13.79)
		Female	0	2(25)	5(62.5)	0	1(12.5)	72±22.22
		Total	0	26(44.06)	30(50.84)	2(3.38)	1(1.69)	63.05(±15.37)
Sistan & Baluchistan	Iranshahr	Male	6(16.66)	19(52.77)	8(22.22)	3(8.33)	0	53.33(±19.96)
		Female	0	5(55.55)	2(22.22)	1(11.11)	1(11.11)	66.66±26.22
		Total	6(13.33)	24(53.33)	10(22.22)	4(8.88)	1(2.22)	56(±21.70)
	Nikshahr	Male	0	4(8.51)	42(89.36)	1(2.12)	0	70.46±7.75
		Female	0	0	20(100)	0	0	72
		Total	0	4(5.97)	62(92.53)	1.(1.49)	0	70.92(±6.51)
	Chabahar	Male	0	7(63.63)	3(27.27)	1(9.09)	0	58.09±6.50
		Female	0	8(61.53)	5(38.46)	0	0	57.23±12.15
		Total	0	15(62.5)	8(33.33)	1(4.16)	0	58(±14)

Discussion

This study was proposed to determine the response of *P. vivax* to chloroquine in standard regimen of treatment in south and southeast of Iran where chloroquine resistance in *P. falciparum* is extensively prevalent (14, 16). The PCT in each studied district showed, more or less, different aspects of the PCT. Although most of PCTs of *P. vivax* in Iranshahr and Chabahar districts occurred at 48 h, most of the PCTs in Bandar- Abbas and Nikshahr districts took place at 72 h. In previous different studies by Edrissian et al. and Hamedi et al. MPCTs in Bandar- Abbas district (Hormozgan Province) were reported as 2.81 and 2.91 d and 67.2 h, respectively (13-15). So there was no significant difference between results obtained in this study 63.05(±15.37) and those by mentioned studies in the district. Such study in Nikshahr and Chabahar (Sistan & Baluchistan Province) was conducted for the first time. The results show that MPCT in Nikshahr district 70.92(±6.51) was higher than the other districts, but still sensitive to chloroquine. MPCTs obtained for male patients were higher than those of female patients in Bandar- Abbas, Iranshahr and Nikshahr districts, but not in Chabahar district (Table 1). The MPCTs for male and female patients in this study were contrary to those found by Edrissian and his colleagues at Bandar-Abbas district (14).

Although chloroguine effectively eliminated the parasites from the studied patients' blood by day four at the most studied areas in Iran, some reports were released from other malarious areas such as Papua New Guinea, Brazil, India and Colombia indicating emergence of chloroquine- resistant strains of *P. vivax* in the areas (8-12). More ever, in this study the parasite clearance time for a number of cases occurred within 96-120 h, and no parasitaemia reappeared in the studied patients after 5 d within the 28-d follow-up period.

In conclusion, although the study showed that chloroquine is still an efficacious drug for the treatment of *vivax* malaria in south and southeast Iran, higher MPCT in Nikshahr district than the other districts indicating this could be an early sign for reduced susceptibility of the parasite to the drug.

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