Original Article

Antimalarial Drug Resistance in Vitro Studies against Plasmodium Falciparum in Punjab, Pakistan

1. Muhammad Saleem Rana 2. Akhtar Tanveer 3. Asma Abdul Latif 4. Ammara Hassan Tahir

1. Assoc. Prof., Health Services Academy, Islamabad 2. Prof. of Parasitology, Punjab University, Lahore
3. Asstt. Prof. of Zoology, Women College University, Lahore 4. Asstt. Prof. of Medicine, AIMC, Lahore

ABSTRACT

Purpose of study: Antimalarial drug resistance was checked against Plasmodium falciparum in Punjab Pakistan.
Study Design: Prospective nonrandomized descriptive study.
Place and duration of study: Study was conducted in seven districts "Muzaffargarh, D.G.Khan, Jhang, Sheikhupura, Faisalabad, Lahore and Multan" of Punjab, Pakistan during the transmission season from July-November of 2003 to 2008.
Materials and Methods: Out of total positive for P.falciparum 612 (228+192+192) subjects were enrolled for study as per eligibility criteria of World Health Organization (WHO) and in vitro standard test kit was used for study. Differences in proportions and its significance were analyzed by chi-square tests.
Result: Resistance (%) to chloroquine was noted higher in males (79.5%) and in females (20.4%). Highest resistance (%) 31.8 was detected in 6-15 years age group. Same resistance (%) trend was observed in basoquine for males (72), females (28.35), for age groups 6-15 years (41.7) and total was 34.8. Sulphadoxine-pyrimethamine found highly effective with only 5.7% resistance. Trend of resistance (%) in male, female and among different age groups was found same in chloroquine and basoquine.
Conclusion: Differences among the resistance (%) sulphadoxine-pyrimethamine and chloroquine or basoquine was highly significant ($p<0.001$) and between basoquine and chloroquine resistance difference was non significant ($p>0.177$). Male of age group 6-15 years having ≥6000 parasite/µl must be treated on priority basis by artesunate combination therapy (ACT). Chloroquine was less effective than sulfadoxine-pyrimethamine (adjusted odds ratio [OR], 6.4; 95% confidence interval [CI], 2.4-17.0; P<.001) and basoquine (adjusted OR, 8.4; 95% CI, 2.0-36.5; P = .004). Chloroquine and sulfadoxine-pyrimethamine were equivalent in efficacy at day 28 (adjusted OR, 1.3; 95% CI, 0.3-7.0; P = .73).

Key Words: Plasmodium falciparum, resistance, sulphadoxine/pyrimethamine, chloroquine, basoquine, Pakistan

INTRODUCTION

Since ancient times, humankind has had to struggle against the pathogenic microorganisms. Among which plasmodium is still the most important pathogenic microorganisms causing malaria worldwide (1). Malaria is a major public health problem; both treatment and control are hampered by the spread of resistance to common antimalarial drugs, especially multi-drug-resistant malaria is highly prevalent (2). Chloroquine resistance in *P.falciparum* contributes to increasing malaria-attributable morbidity and mortality in Sub-Saharan Africa (3). A child dies of malaria every 30 seconds, half of the world's population is at risk of malaria and an estimated 243 million cases led to approximately 863 000 deaths in 2008 (4).

Local strains of *P.falciparum* are resistant to chloroquine and attempt had made to check the susceptibility status of local strains towards chloroquine, basoquine and sulphadoxine-pyremethamine by *in vitro* technique in this study. The efficacy of readily affordable antimalarial drugs is declining rapidly in different parts of the world as reported by (4, 5,6,7,8,9,10,11,12, 13,14,15,16).

MATERIALS AND METHODS

Based upon the Annual Parasite Incidence (API) and/or high proportion of *P. falciparum* compared with *P. vivax* for *in vitro* studies the rural population of the Punjab Province was tested. Out of 6567 subjects screened during the transmission season (July-November of 2003 to 2008), 1406 were found positive for *P.falciparum.* Out of total positive for *P.falciparum* 612 (228+192+192) subjects were enrolled for study as per eligibility criteria of world Health Organization (17). For chloroquine 228 subjects were selected in seven districts (Sheikhupura, Muzaffargarh, Multan, Jhang, D.G.Khan, Faisalabad and Lahore) of Punjab.

Basoquine was tested on 192 subjects in six districts (Sheikhupura, Muzaffargarh, Multan, Jhang, D.G.Khan, and Faisalabad) and sulphadoxine / pyrimethamine on 192 subjects in the same six districts (Figure-1).

Figure-1: Map of Pakistan showing provinces and study districts

When the patient met all the inclusion criteria was enrolled in the study. The results for chloroquine, basoquine and sulphadoxine-pyrimethamine were recorded and conclusions were drawn. W.H.O. *in vitro* standard test kit was used for study. Differences in proportions and its significance were analyzed by using chi-square tests (18).

RESULTS

Chloroquine

228 subjects were treated with chloroquine for uncomplicated *falciparum* malaria in seven districts of Punjab showed 88 (38.6%) resistant cases and malaria incidence was higher among males 168 as compared to females 60 out of total 228 subjects (Figure-2). Total resistant (%) was found 38.6 (88/228), among them male was 30.7 (70/228) and females 7.8% (18/228). It is concluded that male subjects had more tendency to develop resistance as compared to females. Subjects were also analyzed by dividing in three age groups (≤5, 6 to 15 and ≥16 years) to check the treatment failure. Resistance (%) was found 2.19 (5/228) in ≤5, 12.2 (28/228) in 6-15, and 9.2 (21/228) in ≥16 years group. Resistance (%) among the total resistant subjects in different age group was found 5.6 (5/88), 31.8(28/88) and 23.8 (21/88) in age group ≤5, 6 to 15 and ≥16 years respectively. This study concluded that the age group 6-15 years is more vulnerable to develop resistance. The highest resistance (%) 48.1 (40/83) was found in the group having parasite density ≥6000 parasite/µl, 37.9 (22/58) had 3000 to 6000 and 29.8 (26/87) had density ≤3000 parasite /µl. On examination of resistance (%) among total resistance subjects found 22.8 (26/88), 19.4 (22/88) and 35.2 (40/88) in the parasite density /µl group of ≤3000, 3000 to 6000 and ≥6000 respectively. Results showed that chances of resistance increased with the increase of parasite density/ µl.

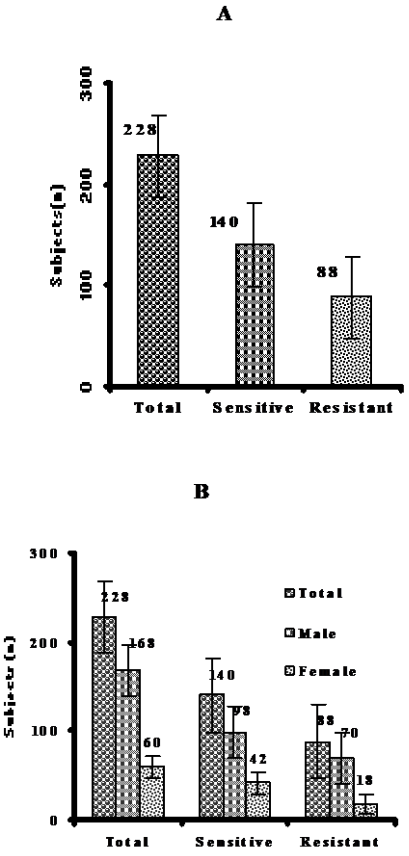
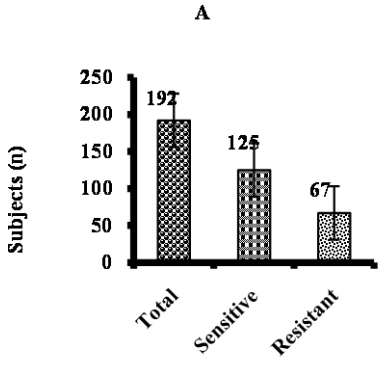


Figure-2 Showing total, sensitive, resistant (A) and male and female (B) subjects by *in vitro* technique against *Plasmodium falciparum* with chloroquine in seven districts of Punjab, Pakistan from July 2003 to November 2008.(adjusted odds ratio [OR], 6.4; 95% confidence interval [CI], 2.4-17.0; P<.001).

Basoquine

192 subjects were treated with basoquine for



uncomplicated *falciparum* malaria in six districts of Punjab and 34.89 (67/192) resistance (%) was found.

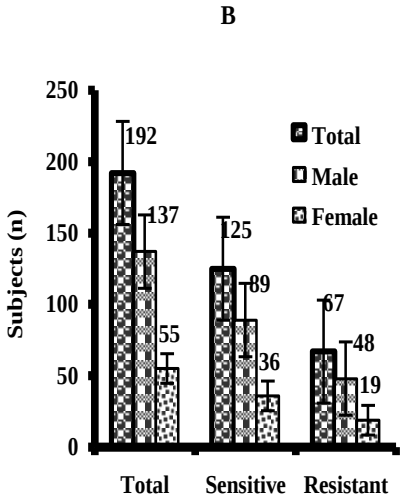


Figure-3 Showing total, sensitive, resistant male and female subjects by *in vitro* technique against *Plasmodium falciparum* with basoquine in six districts of Punjab, Pakistan from July 2003 to November 2005.(adjusted odds ratio [OR], 6.4; 95% confidence interval [CI], 2.4-17.0; P<.001).

The incidence (%) of malaria was higher among males 71.3 (137/192) as compared to female subjects 28.64 (55/192), resistant (%) out of total subjects was found 34.89 (67/192), in males was 25(48/192) and females 9.89 (19/192). Genders wise further analysis of resistance described that out of 67 total resistant subjects, resistance (%) in males was 72 (48/67) and 28.35(19/67) females. There were 137 total male subjects out of them 35% (48/137) were found resistant and among 55 females subjects the percentage of resistant subjects was also 35% (19/55). Data was also analyzed by dividing the 192 subjects in three age groups (≤5, 6 to 15 and ≥16 years) and significant difference of failure was found (p=0.000).

Resistance (%) was found 21.2% (11/192) in ≤5, 53.7% (28/192) in 6 to 15 and 51.8% (27/192) in ≥16 year's old group. Among the same age group subjects resistance (%) was 57.8% (11/19) in 6-15 the resistance (%) was 27.7% (28/101) and in ≥16 year group resistance (%) was 37.5% (27/72). Resistance among total subjects in different age groups was found 6.4% (11/67), 41.8 %(28/67) and 40.3% (27/67) in age groups of ≤5, 6 to 15 and ≥16 years respectively (Figure-3).

Sulphadoxine-pyrimethamine

Sulphadoxine-pyrimethamine was also tested on 192 subjects against un-complicated *falciparum* malaria in seven districts of Punjab and found 5.72% (11/192) resistance (P=. 000[95%CI=. 000-.000], Mean± S.D, 1.9427±. 23301) (Figure-4).

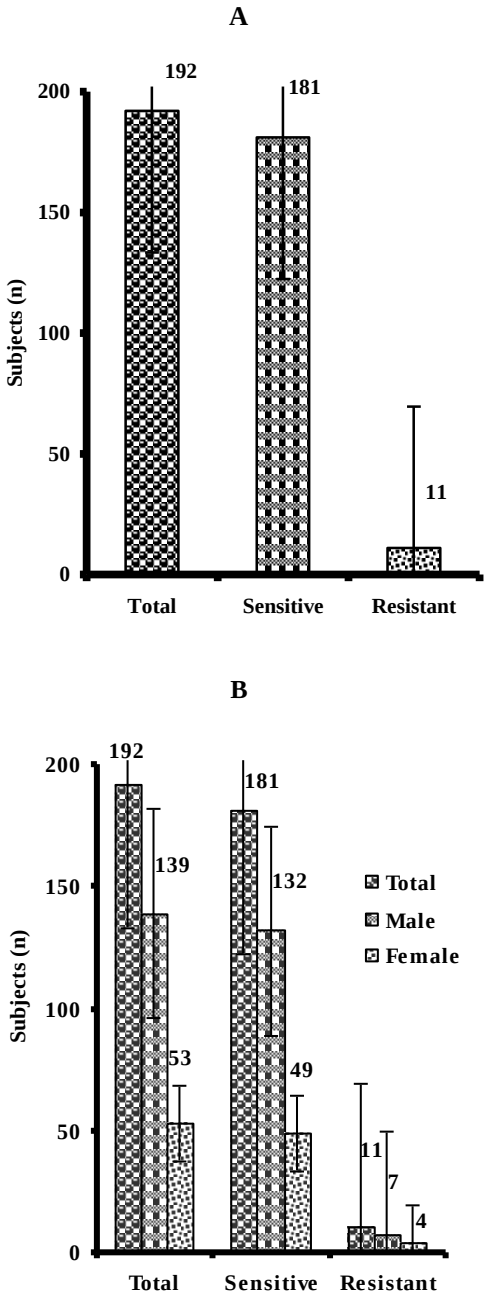


Figure-4 Showing total, sensitive, resistant, male and female subjects by *in vitro* technique against *Plasmodium falciparum* with sulphadoxine-pyrimwethamine in six districts of Punjab, Pakistan from July 2003 to November 2008.(adjusted OR, 1.3; 95% CI, 0.3-7.0; P = .73).

The resistance found against this drug was low and this was might be due to some hidden factors, which could

not be found during this study. The incidence of malaria infection was higher among males 72.3% (139/192) and females 27.6% (53/192). Total resistant (%) was found 5.7% (11/192). Genders wise further analysis of resistance, described that out of total 11 resistant subjects 63.6% (7/11) were males and 36.3% (4/11) were females. Among the same gender, out of total males subjects were 139 out of which 5% (7/139) were found resistant and females subjects were 53 out of which 7.5% (4/53) were *P.falciparum* resistant subjects. No significant difference between male and female were found.

Data was also analyzed by dividing the 192 subjects in three age groups (≤5, 6 to 15 and ≥16 years) and significant difference of failure was found (P=0.005) among three groups for treatment with the same group analysis. Failure percentage was found 5 to 51% highest in age group ≥16 years 63.3% (7/11). Among the total study subjects 192, the young one group (≤5) contributed 0% resistance, 2nd age group had 2% and ≥16 year's group had 3.4% resistant subjects. Parasite density /µl of resistant subjects was found very important factor toward resistance development. The subjects were divided in three parasite density groups (≤ 3000, 3000-6000 and ≥6000 parasite density /µl). In resistant subjects 18.2 % had parasite density ≥6001 parasite/µl. 54.5% (6/11) had 3000 to 6000 and 27.2% had density ≤3000 parasite /µl. Although, the outcome of study is on the limited number of subjects but it concluded that situation is alarming because the incidence of *P.falciparum* and resistance against chloroquine is increasing.

DISCUSSION

A merging multi-drug resistance problem in *P.vivax* and *P.falciparum* malaria parasites in Pakistan and concluded that despite resistance, chloroquine was prescribed in patients with malaria requiring hospitalization (19, 20, 21). We found a high proportion of single antimalarial drug use as well as inappropriate combination therapy (22.7%), and inadequate use of primaquine terminal prophylaxis. Physicians need to be acquainted with malaria treatment guidelines in an endemic zone. In present study chloroquine resistant (%) was found 38.6 by *in vitro* and 35.4 by *in vivo,* in another study conducted in Madagascar (22) resistance (%) detected by *in vitro* was 6.3%.

In this study area wise (Sheikhupura, Muzaffargarh, Multan, Jhang and D.G.Khan) development of resistance (%) detected against chloroquine was 41.6, 50, 41.6, 44.5, and 36.2 respectively. The differences in resistance (%) among different areas were found in the range 0.7 to 15 which is high as compared to difference in resistances (%) among different countries but cumulative difference of resistance (%) was detected 3.7, which does fall in the range 2.6 to 6.3 noted globally. In the same study areas development of resistances (%) against chloroquine and basoquine by *in vitro* technique were detected 41.6, 50, 41.6, 44.5, 36.2 and 33.3, 36.2, 33.3, 30.5, 36.22 in Sheikhupura, Muzaffargarh, Multan, Jhang and D.G.Khan respectively reported by various authors. Resistance differences between two drugs were found of 8.3 to 13.8 which were significant and confirmed that basoquine is more effective in all study areas than chloroquine. In Muzaffargarh and Jhang chloroquine found more resistant as compared to other districts. In D.G.Khan both drugs were found having the same percentage of resistance.

By *in vitro* resistances developed between chloroquine and sulphadoxine-pyremethamine were found 41.6, 50, 41.6, 44.5, 36.2 and 4.1, 8.3, 5.5, 2.7, 5.5 in Sheikhupura, Muzaffargarh, Multan, Jhang and D.G.Khan respectively. Results showed that sulphadoxine-pyremethamine is more effective drug in all study areas than chloroquine. In Muzaffargarh its resistant (%) was comparatively high (8.3) as compared to other districts, which is a matter of concern and cause of it needs to be detected. Basoquine is the antimalarial drug of the same group (4-aminoquinoline) of chloroquine; it is known more effective than chloroquine in the treatment of *P.falciparum* malaria. In the present study basoquine was tested against *P.falciparum* by *in vitro* technique and resistance (%) was found 34.8, which was 3.8% less than chloroquine (38.6), although it is not a big difference but it proved the assumption that it is more effective than chloroquine. Districts wise resistances (%) determined were 33.3, 36.2, 33.3, 30.5 and 36.22 in Sheikhupura, Muzaffargarh, Multan, Jhang and D.G.Khan respectively any significant difference was not found among different study areas.

The highest resistance (%) detected by *in vitro* was 26 and by *in vivo* was 18 to basoquine against *P.falciparum* showed 8% less resistance development by *in vivo* in Kenya (23). The development of resistance (%) to basoquine by day 28 was determined by *in vivo* 22.8 (95% confidence interval) and by *in vitro* 28.7. The difference of resistance (%) found between two techniques was 5.9 in Indonesia which was also similar to present study (24). In Kampala Uganda efficacy of basoquine was assessed by *in vitro* and found resistance (%) 56 in the treatment of uncomplicated *P.falciparum* malaria (25).

In present study by *in vitro* development of resistance (%) against sulphadoxine-pyremethamine was also checked and found 5.7 (11/192) with distribution in different study areas 4.1, 8.3, 5.5, 2.7 and 5.5 in Sheikhupura, Muzaffargarh, Multan, Jhang and D.G.Khan respectively. The resistance (%) detected of sulphadoxine-pyremethamine was less than chloroquine

and basoquine in Punjab, Pakistan. Drug pressure has been recognized to be an important factor in promoting the emergence and propagation of drug resistance, especially in areas with intensive malaria transmission. In Thailand, a decrease in the frequency of *falciparum* malaria was attributed to lower gametocyte carriage rates associated with the introduction of therapy with artimisnin derivatives. Monotherapy of any antimalarial drug should be stopped and switch to combination therapy. A sentinel site surveillance system for surveillance of antimalarial drug efficacy should be established to accomplish the dual goals of timely and ongoing data collection being the best tool.

Acknowledgment: Directorate General Health Services Punjab and Directorate of Malaria Control Pakistan is gratefully acknowledged for their assistance.

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Address for Corresponding Author:
Muhammad Saleem Rana,
Associate Professor,
Health Services Academy,
Islamabad.