PLASMODIUM VIVAX MALARIA: CLINICAL COURSE AND FREQUENCY OF RESISTANCE AGAINST CHLOROQUINE

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ABSTRACT

Objective: To determine frequency of chlorolquine resistant plasmodium vivax and to observe clinical behavior of vivax malaria in our setup.

Study Design: Prospective observational study.

Place and Duration of Study: Combined Military Hospital Tarbela, from Jun 2014 to Jan 2015.

Methodology: One hundred and twenty patients with smear confirmed plasmodium vivax malaria were included. Demographics (age and gender) and response to chloroquine as per epidemiologic definition to resistance were collected in all cases. Patients were observed closely in indoor for any clinical evidence of complications associated with vivax malaria as reported in literature. Frequencies for leucopenia and thrombocytopenia were noted.

Results: Around two third (69%) patients were males with mean age of 27.67 (SD=6.85) years. Two patients showed resistance against chloroquine and rest of the patients had normal response. Seventy percent of patients had thrombocytopenia and 39% had leucopenia. All patients had clinically benign course of illness without any end organ damage.

Conclusion: In patients with plasmodium vivax malaria, chloroquine resistance is infrequent and clinical course is benign in study population.

Keywords: Chloroquine, Malaria, Plasmodium vivax, Resistance.

INTRODUCTION

Human malaria is caused by five known plasmodium species and out of these, *Plasmodium vivax (P.vivax)* is the most widely distributed species and is responsible for 25-40% of malaria cases[1]. Worldwide, incidence of *P. vivax* infections ranges between 130 and 390 million[2,3]. Traditionally, it is considered to be a benign disease, but various studies report significant complications with this infection[4,5]. In addition to commonly encountered upper respiratory tract symptoms, severe lower respiratory complications including acute lung injury and acute respiratory distress syndrome have been described[6,7]. Many cases of splenic rupture have been reported[8,9]. Other serious complications like disseminated intravascular coagulation, shock, renal failure, profound anemia, and cerebral malaria have also been described[10]. The mortality rate for severe infection is generally low, but one report of 36 cases from West Papua, Indonesia reported a death rate of 25%[11]. In addition, hypnozoites of *P. vivax* are well known for causing relapsing disease.

Chloroquine was the first anti-malarial in its class, produced on a large scale for treatment, as well as prevention of malaria infection. It has very good penetration into most tissues which leads to a large volume of distribution. Resultant, serum drug levels are maintained for up to two months[13]. It gets concentrated in digestive vacuole of plasmodia inside the RBCs and prevents polymerization of heme molecules formed during the process of hemoglobin digestion by the parasite. These heme molecules are toxic to the parasite and lead to its killing.

P. falciparum rapidly developed resistance against chloroquine due to its widespread use in most malaria endemic areas leading to dramatic decline in its use and development of alternate regimens. Resistance in *P. vivax* was first time

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reported in 1989, when it was noted that some of repatriates from Papua New Guinea did not respond to standard treatment in Australia[13].

Possibility of resistance to chloroquine is established clinically, as it is difficult to maintain the growth of *P. vivax* in culture and molecular markers of chloroquine resistance have not been well defined.

Epidemiologists have defined chloroquine resistance as persistent parasitemia (e.g. not decreasing) after four days of chloroquine therapy, or recurrence of parasitemia within 28 days following chloroquine therapy in the presence of adequate blood concentrations (>100 ng/mL whole blood concentrations of chloroquine plus its metabolite desethyl-chloroquine)[14]. In some countries, concurrent reports of clinical disease severity and CQ-resistance has led to a study question that there is a possible association between CQ-resistance and clinical severity of vivax disease[16].

This association between chloroquine resistance and clinical severity of *P. vivax* malaria has resulted in the idea of carrying out study in our setup, so that local pattern of chloroquine response and clinical course can be assessed. The results of study can help in estimating chloroquine resistance and clinical course of *P. vivax* disease in our local population.

METHODOLOGY

This prospective observational study was conducted at Combined Military Hospital, Tarbela from June 2014 to January 2015. Sample size was calculated by WHO sample size calculator using 95% confidence interval, absolute precision 6% and anticipated prevalence of 88%[15]. Non probability consective sampling was used. Patients who presented with fever and their Blood films were positive for *P. vivax* trophozoites were included. Patients with following conditions were excluded: (1) History of allergy to the chloroquine; (2) Recent (within past 72 hours) exposure to antimalarial therapy; (3) Co-infection with other plasmodia; (4) Pregnancy; or (5) History of chronic medical conditions like liver, kidney or heart disease. Total of one hundred and twenty subjects fulfilled the study criteria.

Informed consent was obtained from all adults and parents of child participants. The study synopsis was reviewed and approved by the Hospital Ethical Committee.

Treatment: All patients were hospitalized to ensure compliance to the treatment and assess for any tolerance issues. In addition, they were closely observed for any malaria related complications. All were treated with the standard doses of chloroquine (10 mg/kg stat as first Dose and then 5 mg/kg at 8, 24 and 48 hours). Dose was given again to the patients who vomited within 30 min of the drug ingestion. Fever was treated with as required doses of Paracetamol.

Follow up: During admission, the patients were daily followed up clinically to look for any complications associated with severe Malaria such as severe headache with vomiting, shortness of breath, pain abdomen and relevant physical examination. Blood films were also taken to look for presence of Malarial Parasite. Complete Blood Picture and Biochemistry panel were also carried out. Patients were discharged after three days and were further followed up in the outdoor, on day 14 and day 28. And, at every follow up visit, history was taken to look for symptoms of relapse and a blood sample was taken for microscopic examination. Patients, who did not clear parasitemia, were given artemisinin based therapy orally (Artemether with Lumefantrine) as per standard protocol and were not further evaluated.

Outcome: *P. vivax* was categorized into following two categories according to its response to chloroquine treatment:

(1) Sensitive: Microscopically undetectable trophozoites within 72 hours of initiating therapy and at D14 and D28 follow-ups.

(2) Resistant: Microscopically detectable trophozoites at 72 hours after initiating therapy, or if detected on D14 and D 18 follow-ups.

The data was analyzed through SPSS-23. Descriptive statistics were used. Mean and

Standard deviation (SD) was calculated for quantitative variable i.e. age. Percentages were calculated for qualitative variables i.e. gender, thrombocytopenia, leucopenia and chloroquine resistance.

RESULTS

A total of 120 subjects were included in the study. Distribution of gender was males 83 (69%) and females 37 (31%) (table-I). Mean age was 27.67 ± 6.85) years (table-II). Seventy percent of the patients had thrombocytopenia which was severe (platelet count >50 thousands) in 4% (table-III). However, no patient had any bleeding complications. Thirty nine percent had Leucopenia (table-IV), but none had any superadded infection. Two patients (1.7%) showed resistance of *P. vivax* against chloroquine, as evidenced by persistence of fever and positive smear at 72 hours. One hundred and eighteen patients (98%) Cleared parasitemia, were afebrile and smear negative at 72 hours (table-V). None of the patients developed malaria associated serious complications such as shock, cerebral malaria, respiratory distress, spleen rupture, severe anemia, DIC or renal failure.

Table-I: Frequency of gender distribution.

	n (%)
Male	83 (69)
Female	37 (31)

Table-II: Mean age.

	n	Minimum	Maximum	Mean	Std. Deviation
Age	120	12	53	27.67	6.851
Valid N	120	-	-	-	-

Table-III: Frequency of thrombocytopenia.

	n (%)
Platelets >150	36 (30.0)
Platelet 100-150	41 (34.2)
Platelet 50-100	38 (31.7)
Platelet <50	5 (4.2)
Total	120 (100.0)

Table-IV: Frequency of leucopenia.

	n (%)
Normal Leucocyte count	73 (60.8)
Leucopenia	47 (39.2)

Table-V: Frequency of resistance to chloroquine.

Respose	n (%)
Senstive	118 (98.3)
Resistant	2 (1.7)

DISCUSSION

Pakistan has a total estimated population of 195 million and out of this, around 185 million live in malaria endemic areas[15]. There are one million estimated annual malaria cases with *P. vivax* being culprit in 81% and chloroquine is the first line treatment against it[15]. There are various reports of resistance in *P. vivax* against chloroquine and serious complications from around the world[16]. Therefore, it is imperative to evaluate the response of *P. vivax* and also study its clinical severity in this country.

In this study, males are preponderant (male to female ratio 1.77:1), although both genders are equally vulnerable to malaria. This could be explained by the fact that our study setup is a tertiary hospital where majority of the patient are soldiers, whether retired or in service.

In this study, the most common malaria associated complication was thrombocytopenia, seen in 70% of the patients. Severe thrombocytopenia (Platelet count <50000) was seen in 4% of the patients; however none of the patients had abnormal bleeding. Iqbal *et al* found thrombocytopenia in 82% of patients with severe in 14%[17]. Possible underlying mechanisms include macrophage activation, cytokine productions and formation of antiplatelet globulins leading to accelerated platelet destruction. Other possibilities include oxidative stress, platelet sequestration in non-splenic sites and clumping. In this study, frequency of leucopenia was 39%, but no patient had any superadded infection. A study by Rasheed *et al* found neutropenia in 21% and thrombocytopenia in 75% of the patients[18]. Naha *et al* found leucopenia in 38% of their cases and thrombocytopenia in 86%[19].

In this study, less than two percent of the patients showed clinical resistance to Chloroquine only and no patient had relapse up to 28 days of follow-up. Furthermore no patient had any malaria related serious complications like severe anemia, ARDS, splenic rupture etc. Waqar *et al* found chloroquine to be 100% effective in *P. vivax* and no malaria related complications[20]. Leslie *et al* found 1.3% of *P. vivax* were resistant to chloroquine[21]. Subhani *et al* found relapse in 6% of their study patients but again without any malaria related complication[22]. However in 2013, the indirect evidence of chloroquine resistance has been revealed by the fact that *P. Vivax*. Carrying pvmdr1 codon F1076L mutation is prevalent which is a marker of resistance to chloroquine and primaquine[23]. A single case of clinically confirmed chloroquine resistance carrying mutant pvdhfr and pvdhp genes has also been reported by Waheed *et al*[24]. This study is unique in this aspect that epidemiologic data regarding chloroquine resistant *P. vivax* in Pakistan are scarce, even in the most recent global analysis[16].

This study has been carried out in a small set up with relatively small number of patients. Patients don't represent the local area population nor do they represent local pattern of chloroquine resistance as army patients belong to different areas of Pakistan. No complications might have been due to early detection and timely treatment of the patients due to well established medical setup of the army, which is not the case in health-care facilities in public Set up[25]. Large scale studies are required at various regions to establish the exact incidence of resistance in *P. vivax* and the clinical course of malaria caused by this organism.

CONCLUSION

In patients with plasmodium vivax malaria, chloroquine resistance is infrequent and clinical course is benign, in this study. Clinicians treating malaria patients should remain alert for primary failure of chloroquine treatment, though.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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