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Plasmodium vivax Polymorphism in a Clinical Drug Trial

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Data from a double-blind randomized clinical drug trial were analyzed to find the comparative responses of two antirelapse drugs, bulaquine and primaquine, against different relapsing forms of *Plasmodium vivax* infection. A 1-year follow-up study strongly suggests that the duration of preerythrocytic development of *P. vivax* is a polymorphic characteristic, exhibited by two strains of hypnozoites responsible for early and late manifestations after primary infection. Short-term relapses were significantly higher in the first half year than long-term relapses, and the reverse was true in the second half year. Clinical drug response data showed that the hypnozoites characterized for short-term relapse were not susceptible to either of the antirelapse drugs in the currently administered dose, whereas hypnozoites characterized for long incubation were significantly susceptible.

Plasmodium vivax, the predominant species of malaria parasite, has a relapse mechanism that results in the reappearance of parasitemia arising from the preerythrocytic hepatic-stage hypnozoite (13) which develops at characteristic predetermined intervals following primary infection. It is well known that *P. vivax* exhibits two primary types of relapse pattern, apparently depending on the geographical origin of the parasites. The typical Chesson strains, referred to as the tropical-zone type, from the South Pacific and Southeast Asian regions (New Guinea-South Pacific), are characterized by early primary attack followed by a short latent period before the appearance of frequent relapses, whereas strains that originate in temperate zones (e.g., St. Elizabeth [United States]) exhibit early primary attack followed by a long latent period of 6 to 14 months succeeded by a series of relapses at short intervals (6, 7, 10).

Although therapeutic application of primaquine, an 8-aminoquinoline drug, is restricted due to its reported adverse toxicological side effects (3, 4, 8, 23), it is the only drug in clinical use for the prevention of relapsing *P. vivax* infection. Recently, the comparative clinical efficacy of a new primaquine derivative, bulaquine (9), versus primaquine in preventing relapse in vivax malaria has been reported (24). The present report is an attempt to analyze further the responses of bulaquine and primaquine against different parasite forms of *P. vivax* responsible for different relapse patterns.

MATERIALS AND METHODS

Patient selection. The study was approved by the Ethical Committee of the Central Drug Research Institute, Lucknow, India. Before enrollment in the study, written informed consent was obtained from all patients. Patients reporting to the clinic for the first time (having no history of malaria) with acute illness and showing symptoms such as high fever, severe headache, loss of appetite, occasional vomiting, and microscopic evidence of *P. vivax* infection were considered primary cases and were enrolled in the clinical trial according to exclusion and inclusion criteria (24).

The patients enrolled in the study were instructed not to take any drugs, including antimalarials from other sources, and the movements of these patients were strictly monitored. The patients were also advised to take personal protection measures to avoid possible reinfection during the study period.

Treatment and follow-up. All patients enrolled in the trial were treated with 1,500 mg of chloroquine base in divided doses over 3 days. Subsequently, on day 4, the patients were assigned to one of the three treatment groups, i.e., placebo, primaquine, or bulaquine, in a double-blind manner (24). Primaquine (15 mg once a day), bulaquine (25 mg once a day), and a placebo were given orally for 5 days following the chloroquine therapy (24). Of 663 enrolled patients, 571 were followed up fortnightly by active surveillance for 1 year.

Classification of relapse versus nonrelapse. The drug code for each patient was unblinded after completion of a 1-year follow-up, and the patients were classified into relapse and nonrelapse groups. Patients who had no clinical symptoms of malaria or parasitological evidence of *P. vivax* infection during the entire study period following their primary infection and subsequent enrollment were considered nonrelapse cases. Those patients who reported back to the clinic or were found during active surveillance within 1.5 months to 1 year to have renewed clinical symptoms (mild) along with a periodic alternate-day fever (not observed in the primary cases) and were found to be microscopically positive for *P. vivax* infection were considered relapse cases. It should be pointed out that in relapse cases the clinical symptoms observed were noticeably milder than in primary cases, in which they were acute. In addition, the periodicity of fever in relapsed patients was typically tertian from the very onset of the infection, probably due to synchronization of the parasite's asexual cycle. On the other hand, the periodicity of fever was conspicuously absent in the primary cases. However, some uncertainly still exists and will probably persist until a diagnostic tool is developed to distinguish primary infection from relapse cases. The time interval between the primary attack (the date of enrollment in the trial) and the first relapse (the date of the second attack) was calculated as lag months: 30.4 days was considered to be 1 month, 0.5 months to <1.50 months was considered 1 lag month, >1.5 months to <2.50 months was considered 2 lag months, and so on.

Statistical analysis of data. The clinical efficacies of the two antirelapse drugs were estimated by comparing the relapse rates observed after 1-year follow-up of patients in each drug and placebo group (24). A similar comparison was also made between two categories of patients, i.e., those relapsed within 6 months (short term) and those relapsed between 6 and 12 months (long term). Subsequently, relapse phenomena in different groups of patients were correlated with their registration periods, i.e., between January and June (low-transmission season; first half year) and between July and December (high-transmission season; second half year). To test the differences in antirelapse efficacy, standard chi-square analysis and a normal deviate test (*c* test) were performed (15).

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TABLE 1. Relapse rates in different treatment groups in 1-year follow-up

Group	Total no. of patients	No. (%) of nonrelapse patients	No. (%) of relapse patients
Bulaquine (a)	219	154 (70.32)	65 (29.68)
Primaquine (b)	220	161 (73.18)	59 (26.82)
Placebo	224	134 (59.82)	90 (40.18)
Combined (a + b)	439	315 (71.75)	124 (28.25)

RESULTS

The relapse rates for two antirelapse drugs, primaquine and bulaquine, were 26.82 and 29.68%, respectively (Table 1), after 1 year of follow-up (24) and were not found to be significantly different from each other ($\chi^2 = 0.44$; $0.7 > P > 0.5$). Hence, the data were pooled, and the relapse rate of the combined-treatment group (28.25%) compared with the relapse rate in the placebo group (40.18%) was found to be highly significant ($\chi^2 = 9.66$; $0.01 > P > 0.001$), which indicates that both these drugs have significant antirelapse efficacy compared to the placebo.

To analyze the drug response against different incubating forms of *P. vivax,* relapse patients were regrouped (Table 2) based on their lag month intervals, i.e., patients relapsing within 6 months (short term) and those relapsing between 6 and 12 months (long term). The short-term relapse rates in the primaquine and bulaquine groups were 16.36 and 15.53%, and the long-term relapse rates were 10.46 and 14.16%, respectively, which did not differ significantly from each other ($\chi^2 = 0.05$ [$0.9 > P > 0.8$] and $\chi^2 = 1.39$ [$0.3 > P > 0.2$]). Hence, these treatment groups were combined and used for further analysis. Chi-square test analysis of short-term relapse rates in the placebo (18.75%) and combined-treatment (15.95%) groups did not show any significant difference ($\chi^2 = 0.83$; $0.4 > P > 0.3$), indicating no therapeutic effect against short-term relapsing cases. In contrast, the differences against long-term relapses (21.43 versus 12.30%) were found to be highly significant, ($\chi^2 = 9.49$; $0.01 > P > 0.001$), suggesting significant antirelapse efficacy against long-term relapsing cases.

To find the seasonal prevalence of short- and long-term relapses and the frequency distribution and ratios of these two parasite forms, the patients were regrouped (Table 3) into two categories, those enrolled in the first half year, i.e., between January and June (low-transmission season) and those enrolled in the second half year, i.e., between July and December (active-transmission season). In the placebo group, short-term and long-term relapses were 32.65 and 4.08%, respectively, in the first half year compared to 14.86 and 26.29% in the second half year. The difference estimated by the chi-square test was found to be highly significant ($\chi^2 = 15.40$; $P < 0.001$), suggesting that the rate of short-term relapses was significantly higher in the first half year, while that of long-term relapses was significantly higher in the second half year.

TABLE 2. Short- and long-term relapse rates in different treatment groups

Group	Total	No. (%) of patients			
		1–6 lag mo (short term)		7–12 lag mo (long term)	
		Nonrelapse	Relapse	Nonrelapse	Relapse
Bulaquine (a)	219	185 (84.47)	34 (15.53)	188 (85.84)	31 (14.16)
Primaquine (b)	220	184 (83.64)	36 (16.36)	197 (89.55)	23 (10.46)
Placebo	224	182 (81.25)	42 (18.75)	176 (78.57)	48 (21.43)
Combined (a + b)	439	369 (84.05)	70 (15.95)	385 (87.70)	54 (12.30)

Short- and long-term relapses observed with bulaquine and primaquine treatment did not differ significantly during the first ($\chi^2 = 4.82$; $0.1 > P > 0.05$) and the second ($\chi^2 = 0.83$; $0.7 > P > 0.6$) half years. Further, short- and long-term relapses observed in the combined-treatment group also did not differ significantly from those in the placebo group ($\chi^2 = 0.40$; $0.9 > P > 0.8$) in the first half year. However, in the second half year, the difference was found to be highly significant ($\chi^2 = 12.02$; $0.01 > P > 0.001$). Further, data analysis was done to find the effect of treatment based on the comparison of first and second half year data for short-term relapses. The hypothesis of no effect of an antirelapse drug as judged by comparison of short-term relapses in the combined-treatment group versus the placebo group (27.91 versus 32.65%, respectively, during the first half year and 12.75 versus 14.86% during the second half year) was found tenable (nonsignificant *c* values, 0.57 and 0.66). However, a significant difference in the *c* value (2.94) was noticed when short-term relapses in the first half year (27.91%) were compared to those in the second half year (12.75%) in the treatment group. A similar significant difference was noticed among short-term relapses when the relapse rates observed in the placebo group in the first and second half years (32.65 and 14.86%) were compared. Further, no significant difference was noticed between long-term relapses in the first half year in the treatment and placebo groups (3.49 versus 4.08%; *c* value, 0.171); however, in the second half year, a significant difference was noticed (14.73 versus 26.29%; *c* value, 3.02). The difference in long-term relapses in the first and second half years within the treatment group (significant *c* value, 4.11) clearly suggests that treatment had a significant effect only in reducing long-term relapses and probably had less or no effect on short-term relapses. The results of chi-square and normal deviate (*c*) tests of the hypothesis, presented in Tables 1 to 3, are summarized in Table 4.

DISCUSSION

A randomized, blinded, placebo-controlled clinical drug trial was undertaken to find the comparative antirelapse efficacy of bulaquine versus the conventional 5-day primaquine regimen presently practiced by the National Anti-Malaria Programme in India. The results revealed that both are partially effective in preventing relapses compared to a placebo, assuming all patients would have had relapses (in 1-year follow-up) if radical treatment had not been given (24). Further classification of relapsed patients based on their incubation intervals revealed two distinct relapse patterns in the present study: group I, conforming to the tropical, or Chesson strain, type of relapsing *P. vivax* infection with a short period of latency between the primary attack and the first relapse, i.e., short-term relapse, and group II, conforming to the temperate, or St. Elizabeth strain, type that has a long period of latency between the primary attack and the first relapse, i.e., long-term relapse (6, 7). It should be mentioned that the existence of both tropical-

TABLE 3. Seasonal prevalence of short- and long-term relapses in three treatment groups

Treatment	No. (%) of cases				
	Total	Nonrelapse	Relapse	Relapse period of:	
				<6 mo (short term)	>6 mo, <12 mo (long term)
First half (January to June)					
Bulaquine (a)	40	24	16	13 (32.5)	3 (7.5)
Primaquine (b)	46	35	11	11 (23.91)	0 (0.00)
Combined (a + b)	86	59	27	24 (27.91)	3 (3.49)
Placebo	49	31	18	16 (32.65)	2 (4.08)
Second half (July to December)					
Bulaquine (a)	179	130	49	21 (11.73)	28 (15.64)
Primaquine (b)	174	126	48	25 (14.37)	23 (13.22)
Combined (a + b)	353	256	97	45 (12.75)	52 (14.73)
Placebo	175	103	72	26 (14.86)	46 (26.29)

and temperate-zone strains of *P. vivax* in Delhi has been reported (1). It was interesting that the relapse rates observed in control, individual-treatment and combined-treatment groups within a 6-month observation period were similar, as if there was no effect of treatment, whereas beyond 6 and within 12 months of observation, significant antirelapse action was noticed. These two groups of relapses, viz., short term (relapsing within 6 months) and long term (relapsing between 6 to 12 months), are probably due to the existence of two strains of hypnozoites characterized for short and long incubation intervals and having differential responses to antirelapse drugs. In view of this differential drug response observed in the present analysis, it is suggested that the hypnozoites for short-term relapse are probably nonsensitive to the antirelapse drugs or more tolerant of the presently administered dose (primaquine at 15 mg/day for 5 days), whereas hypnozoites with a long incubation period are sensitive to both antirelapse drugs.

It is well known that the response of malaria parasites to drugs depends not only on the species but also on the strain within the same species; thus, some strains have an inherent degree of drug tolerance, and treatment requires a much higher drug dosage than for other strains. In this regard, a number of studies have shown a heterogeneity in strains and geographic isolates of *P. vivax* by a variability of doses of 8-aminoquinolines required to prevent relapses. For example, the Korean strains of *P. vivax*, a temperate-zone type, were reported to be highly sensitive to 14 days of treatment with 15 mg of primaquine (2), while for an unfailing radical cure of the Chesson strain, a tropical-zone type, 30 mg of primaquine daily for 14 days should be given (5).

The occurrence of relapse in *P. vivax* infection after 5-day primaquine therapy (15 mg/day) evident in the present study strongly suggests that the present 5-day drug regimen does not prevent relapse effectively, and higher dosages of primaquine or bulaquine are probably required. In this context, it should be pointed out that *P. vivax* strains from Southeast Asia exhibited a tendency to cause relapse earlier and more often than many other strains; hence, higher dosages of primaquine are probably required to effect a radical cure (12). It should be mentioned that since 1953, the Indian National Anti-Malaria Programme has administered 15 mg of primaquine for only 5 days based on reports of only 5 to 10% breakthrough (20, 22). However, the concept of only 5 to 10% breakthrough is no longer valid, as clinical evidence demonstrating efficacy of the 5-day regimen of primaquine is lacking, as is also evident in the present study. The occurrence of relapse in *P. vivax* infection

TABLE 4. Test of significance in relapse rates among different groups

Groups compared	χ^2 or c value[a]	P value	Table no.
Primaquine vs bulaquine (1-yr follow-up)	$\chi^2 = 0.44$ (df = 1)	$0.7 > P > 0.5$	1
Combined treatment vs placebo (1-yr follow-up)	$\chi^2 = 9.66*$ (df = 1)	$0.01 > P > 0.001$	1
Short term in primaquine vs bulaquine (within 6 mo)	$\chi^2 = 0.05$ (df = 1)	$0.9 > P > 0.8$	2
Long term in primaquine vs bulaquine (within 7–12 mo)	$\chi^2 = 1.39$ (df = 1)	$0.3 > P > 0.2$	2
Short term in combined vs placebo (within 6 mo)	$\chi^2 = 0.83$ (df = 1)	$0.4 > P > 0.3$	2
Long term in combined vs placebo (within 7–12 mo)	$\chi^2 = 9.49$ (df = 1)	$0.01 > P > 0.001$	2
Short and long in placebo, first half yr vs second half yr	$\chi^2 = 15.40*$ (df = 2)	$P < 0.001$	3
Short and long in bulaquine vs primaquine, first half yr	$\chi^2 = 4.82$ (df = 2)	$0.1 > P > 0.05$	3
Short and long in bulaquine vs primaquine, second half yr	$\chi^2 = 0.83$ (df = 2)	$0.7 > P > 0.6$	3
Short and long in combined vs placebo, first half yr	$\chi^2 = 0.40$ (df = 2)	$0.9 > P > 0.8$	3
Short and long in combined vs placebo, second half yr	$\chi^2 = 12.02*$ (df = 2)	$0.01 > P > 0.001$	3
Short term in combined vs placebo, first half yr	$c = 0.57$	$0.60 > P > 0.50$	3
Short term in combined vs placebo, second half yr	$c = 0.66$	$0.50 > P > 0.40$	3
Short term in combined, first half yr vs second half yr	$c = 2.94*$	$0.01 > P > 0.001$	3
Long term in combined vs placebo, first half yr	$c = 0.17$	$0.9 > P > 0.8$	3
Long term in combined vs placebo, second half yr	$c = 3.02*$	$0.01 > P > 0.001$	3
Long term in combined, first half yr vs second half yr	$c = 4.11*$	$P < 0.001$	3

[a] *, significant; df, degrees of freedom.

after 5 days of primaquine therapy strongly indicates the resistance at least of the tropical-zone type of *P. vivax;* therefore, the present drug policy of the National Anti-Malaria Programme for the administration of primaquine for radical cures of *P. vivax* infection warrants an urgent review. In light of our experience, we feel strongly that higher doses of primaquine should be given for the radical treatment of *P. vivax* infection.

In the last decade, many natural polymorphic characteristics, viz., isozyme types (11), drug resistance (16, 18, 21, 25), and antigen composition (17, 19), have been reported in *P. vivax* from many parts of the world. The duration of the preerythrocytic development of *P. vivax* has also been hypothesized to be a polymorphic characteristic (14). However, how these polymorphic characteristics affect the clinical manifestations and epidemiology of *P. vivax* malaria is yet to be investigated. The present clinical drug trial data strongly suggest that the duration of preerythrocytic development is a polymrophic characteristic, which is exhibited by the existence of at least two types of hypnozoites. In view of the differential drug response of *P. vivax,* as evident in the present study, it is suggested that the seasonal ratios and frequencies of these two hypnozoites responsible for different durations of preerythrocytic development should be determined in different *P. vivax* ecosystems, which will help in understanding the transmission dynamics of *P. vivax* and will have direct relevance in implementing any future malaria control strategies and future vaccine action programs.

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