Correlation Between *Plasmodium vivax* Variants in Belém, Pará State, Brazil and Symptoms and Clearance of Parasitaemia

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The aim of this study was to determine how different types of *P. vivax* affect clinical symptoms and parasitaemia clearance. Blood was collected from individuals from Pará State, Brazil. The patients were treated as chloroquine plus primaquine. *P. vivax* were typed daily till D7 and again on D30. Now we can confirm a previously reported correlation between *P. vivax* genotype and response to chloroquine. Clinical symptoms do not allow for objective identification of different *P. vivax* types in the Brazilian Amazon, since the VK247 and *P. vivax*-like have only been detected in mixed infections.

Key Words: *Plasmodium vivax* variants, symptoms, chloroquine and primaquine treatment, clearence of parasitaemia.

Plasmodium vivax has been the most common cause of human malaria in the Brazilian Amazon region during the last seven years. Its variants (VK210, VK247 and *P. vivax-like*) are found in mixed infections; VK210 has also been found as a single infection [9]. Reduction in susceptibility to chloroquine has been reported from Papua New Guinea, India, Asia and South America [1,13,19], though no relationship between *P. vivax* genotypes and parasite clearance following treatment with chloroquine has been found. Kain et al. (1993) suggested that response to chloroquine varies depending on the type of *P. vivax*, since the VK210 genotype and mixed infection with VK247 took longer to clear, while VK247 tended to have a shorter duration in Thailand. A study conducted in Brazil showed no significant difference in the time of parasite clearance after treatment with chloroquine and primaquine, alone or combined [9]. Variants of *P. vivax* can produce different clinical signs and responses to treatment [5], as there is a correlation between *P. vivax* genotypes and the intensity of symptoms and vector preference, which can affect drug resistance and consequent failure of control measures [4]. We examined how different types of *P. vivax* affect clinical symptoms and parasitaemia clearance.

Blood was collected from 30 individuals from Belém city (Pará State) who had signed the informed consent form. The blood samples were obtained before therapy was initiated (D0), and continued daily till day 5 (D5). Vacutainer tubes containing EDTA (Becton Dickinson, UK) were used to collected 5 mL of whole blood/ individual that was subsequently applied to glass-fiber-membrane discs (Titertek, ICN Biomedicals Limited, UK) following the protocol described by Warhurst et al. [21]. The patients were treated as follows: 25 mg chloroquine/kg body weight during 3 days (10 mg/kg on day 1 and 7.5 mg/kg on days 2 and 3), plus 0.25 mg primaquine /kg for 14 days, starting on the fourth day. *Plasmodium vivax* types were identified by

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GFM/PCR/ELISA [9] for all samples. The patients were evaluated daily by a physician till D7, and again on D30.

The typing of *P. vivax* genotype in D0 detected 16 samples with a single infection, all by VK210 (53%) and 14 samples with mixed infections (47%): four VK210 plus VK247 (13%), two VK210 plus *P. vivax-like* (7%), five VK247 plus *P. vivax-like* (17%) and three with all variants (10%). After 24 hours of treatment (D1), VK247 was no longer detectable in one sample (VK210 plus VK247), nor was *P. vivax*-like found in two samples (VK247 plus *P. vivax*-like and VK210, VK247 and *P. vivax*-like). At D2 (48 hours of treatment), VK247 was not detectable in another mixed sample (VK210 plus VK247). After 72 hours of treatment (D3), we observed VK210 in 56% of the pure infection samples and in 67% of the mixed ones, VK247 in 17% and the *P. vivax-like* variant in 40% ($p<0.05$ - X^2 test). All samples were negative in D4. No patient had progressed to relapse at 30 days after treatment.

The clinical signs and symptoms at D0 were similar to those previously described [2,10], and significant correlations ($p<0.05$ - X^2 test) between symptoms and *P. vivax* genotypes were found only for myalgia and splenomegaly. Myalgia was found in 11 patients infected by VK210 alone and in seven patients with mixed infection (one by VK210 plus VK247, one by VK210 plus *P. vivax-like* and five by VK247 plus *P. vivax*-like). Splenomegaly was observed in only one patient with mixed infection, by VK210 plus *P. vivax*-like. We found no significant signs or symptoms for any of the *P. vivax* genotypes, from D2 on.

Variety VK210 was the most frequent in Belém, and it was the only genotype found as a single infection, while VK247 and *P. vivax-like* variants continued to be found only as mixed infections. There have been no significant ($p<0.05$ - X^2 test) changes in the frequencies of *P. vivax* variants in Belém for the last four years, as these frequencies were similar to those found in a previous study [9]. This fact suggests that there have been no ecoepidemiological changes, nor was there enough time for VK247 and *P. vivax-like* to adapt to this region, which was expected since no new species of anophelines have been introduced into Belém (Póvoa et al., in press). Rodriguez et al. (2000) observed a significant correlation between the variant frequency and mosquito susceptibility in Mexico. Our results on the response to chloroquine treatment are similar to those found by Kain et al. (1993), who found a significant correlation between parasitaemia clearance and *P. vivax* genotypes (VK210 and VK247). We found the same correlation for the *P. vivax*-like variant. The difference in the results on the correlation between chloroquine treatment and *P. vivax* genotypes between the previous study made in Belém [9] and our study, is that in our study primaquine was only introduced on the fourth day of treatment and *P. vivax* typification was done daily. Now we can confirm a previously reported correlation between *P. vivax* genotype and response to chloroquine. As we observed no patient relapse at D30, we believe that this treatment continues to be effective for *P. vivax* strains in Belém.

Clinical symptoms do not allow for objective identification of different *P. vivax* types in the Brazilian Amazon, since VK247 and *P. vivax*-like have only been detected in mixed infections. However, we found that VK210 is the type most strongly correlated with the classic symptoms of *P. vivax* malaria. These results could be a consequence of differences in the emergence of each genotype in this geographical region.

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