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SHORT REPORT: USEFULNESS OF THE *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER T76 GENOTYPE FAILURE INDEX FOR THE ESTIMATION OF *IN VIVO* CHLOROQUINE RESISTANCE IN BURKINA FASO

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Abstract. The prevalence of chloroquine (CQ) treatment failure and the genotype failure index was determined in four sentinel sites in Burkina Faso. In three sites, the genotype failure index varied between 1.7 and 3, a result confirming the relationship between the *Plasmodium falciparum* CQ resistance transporter (*Pfcrt*) T76 mutation and CQ resistance. In the remaining site, the genotype failure index was unusually low, 1.1, which was significantly different than that in the other sites ($P < 0.00001$). These findings are discussed. Often but not always, the prevalence of CQ resistance can be correctly estimated by the *Pfcrt* T76 genotype failure index.

The amount of chloroquine (CQ) resistance is usually estimated by carrying out World Health Organization (WHO) *in vivo* tests. However, this is a time-consuming task and results are difficult to interpret where re-infection is frequent.[1] The identification of the relationship between the *Plasmodium falciparum* CQ resistance transporter (*Pfcrt*) T76 mutation and CQ resistance resulted in a series of field studies that investigated the usefulness of this molecular marker in estimating CQ resistance.[2–5] Data from Mali suggest that its prevalence is 2–3-fold higher than *in vivo* CQ resistance.[1] Two indexes, the genotype resistance index (GRI) and the genotype failure index (GFI), which correspond to the ratio between the age-adjusted frequency of the *Pfcrt* T76 mutation and the prevalence of parasitologic or clinical failure, respectively, were defined. They were proposed as useful tools to estimate, over large areas and without the need of carrying out expensive and time-consuming *in vivo* tests, CQ resistance, at least when it is less than 30–50%. However, the GRI and the GFI have not been applied or validated extensively. We therefore computed, based on the prevalence of the *Pfcrt* T76 mutation, the GFI in four sites in Bobo Dioulasso, Burkina Faso in two different years (1998 and 2001) and related it to the prevalence of CQ resistance estimated by *in vivo* 14 days test in 1998–1999 and 2000–2001.

This is part of a larger study that investigated CQ and sulfadoxine-pyrimethamine efficacy in children between 6 months and 15 years of age. This study was reviewed and approved by institutional Ethical Board at Centre Muraz. Informed consent for participation in the study was obtained from the children's parents or guardians. In the following analysis, only CQ efficacy has been considered. Details of the study methodology have been reported elsewhere.[6] Outcomes were defined according to the WHO modified classification for monitoring antimalarial drug resistance.[7] Total treatment failure (TTF) was defined as the sum of early treatment failure (ETF), late clinical failure (LCF), and late parasitologic failure (LPF).

Blood samples for the molecular analysis were collected on no. 3 filter paper (Whatman, Maidstone, United Kingdom) at day 0 before treatment. The DNA was extracted using the Chelex-100 method.[8] Detection of the *Pfcrt* T76 mutation was done by using a polymerase chain reaction, followed by-sequence-specific restriction enzyme digestion (http://www.medschool.umaryland.edu/CVD/plowe.html). The GFI was computed by dividing the frequency of the *Pfcrt* T76 mutation by the prevalence of TTF.

Between 1998 and 2001, a total of 1,068 patients were enrolled in the four sites. The mean age of the children included in the study was similar in the four study sites and varied between 51.7 months in Lena in 1998–1999 to 52 months in Toussiana in 1998 and 2001. Outcome was known for 884 (83%) patients who completed the follow-up. The TTF increased from 20.4% (69 of 337) in 1998–1999 to 28.7% (157 of 547) in 2000–2001 (odds ratio [OR] = 0.6; 95% confidence interval [CI] = 0.4–0.9, $P = 0.0006$), and was composed of mostly LCF or LPF. Such an increase occurred in all sites, although the difference between the two periods was statistically significant (OR = 0.5, 95% CI = 0.2–0.9, $P = 0.03$) at only one site (Lena).

Seven hundred sixteen pre-treatment blood samples (340 in 1998 and 376 in 2001) were randomly selected and analyzed for polymorphism in the *Pfcrt* 76 locus. In all sites, the prevalence of *Pfcrt* T76 mutation increased between 1998 and 2001 (Figure 1), but the difference between the two years was statistically significant only in Accart-ville (OR = 0.5, 95% CI = 0.3–1, $P = 0.03$). In Bama, the prevalence of the mutation was low (22% in 1998 and 33% in 2001) and significantly different from the other sites ($P < 0.00001$ for both years). In three sites (Accart-ville, Lena, and Toussiana) the prevalence of the *Pfcrt* T76 mutation was always higher than the TTF (Figure 1) and the GFI ranged between 1.7 and 3. However, in both years in Bama, the prevalence of the *Pfcrt* T76 mutation was almost identical to that of the TTF with a GFI of 1.1.

The GFI has been proposed, on the basis of data from Mali showing its stability at sites with different levels of transmission, levels of immunity, and ethnic composition, as a simple and reliable tool to monitor CQ resistance in places where this is less than 30–50%.[1] We found similar results in most but

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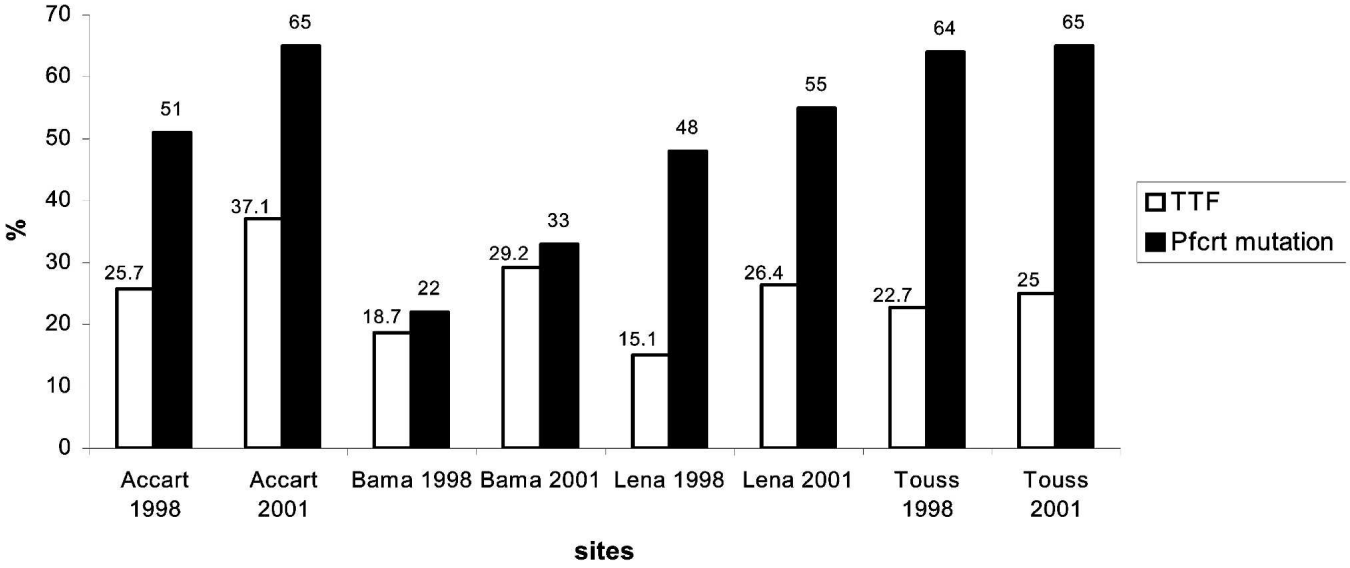

FIGURE 1. Prevalence of the *Plasmodium falciparum* chloroquine resistance transporter (Pfcrt) T76 mutation and *in vivo* chloroquine resistance in 1998 and 2001 in four study sites in Burkina Faso. TTF = total treatment failures; Accart = Accart-ville; Touss = Toussiana.

not all our study sites. The GFI in Accart-ville, Lena, and Toussiana ranged between 1.7 and 3, and was constant over the study period. However, in Bama we observed a low prevalence of the *Pfcrt* T76 mutation, a result that was different from those reported by other studies in west Africa.[9–12] Similar finding have been reported in a study carried out in Madagascar where only 3 of the 43 isolates were resistant to CQ *in vitro* and none carried the mutation.[13] The results from Bama are unexpected because the prevalence of CQ resistance is approximately 20–30%, which is similar to that reported in previous studies,[6,14] and also similar to the prevalence of the *Pfcrt* T76 mutation, which resulted in a GFI of 1.1 for both years studied. An overestimation of CQ resistance can be ruled out because the slides were regularly subjected to quality control in all four sites. The mean age of patients was also comparable between the four study sites, thus ruling out a possible role of the acquired immunity in explaining the difference observed. A possible explanation of different GFIs at various sites is that CQ resistance has a multigenic basis in which the *Pfcrt* T76 mutation is necessary but not sufficient.[15]

Another specific and selective factor in Bama is the much higher frequency of *Anopheles gambiae* M (Mopti) compared with the other sites where both forms M and S (Savanna) occur.[16] High transmission has been associated with a faster spread of CQ resistance, once this has emerged.[15] In Uganda, CQ resistance seems to spread faster, regardless of drug pressure in low and high transmission areas.[17] In Bama, where malaria transmission is high, this does not seem to happen. The high frequency of *An. gambiae* M could be involved in this phenomenon, although it is not clear how.

A major limitation of this study is that we observed a GFI close to 1 only in one site, although the study was conducted in two different years (1998 and 2001). In the other three sites, estimating the amount of CQ resistance by dividing the prevalence of the *Pfcrt* T76 mutation by 2 or 3 seems to confirm the usefulness of the GFI.[1] It would be important to determine whether this is still the case in areas of extremely high transmission, such as Bama.

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