Relapse of imported vivax malaria despite standard-dose primaquine therapy: an investigation with molecular genotyping analyses

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Abstract

Taiwan CDC investigated four cases of recurrent imported vivax malaria during 2003–2010. Molecular genotyping results and the lack of inter-episodes travel history indicated that two of the patients, who acquired vivax malaria in Indonesia and the Solomon Islands, respectively, suffered relapses after an interval of 3–4 months, despite completing standard-dose primaquine therapy (30 mg/day for 14 days) for the first episode. Treatment with a higher dose of primaquine (60 mg/day for 14 days) prevented further relapse in both patients. This finding calls for further monitoring of the therapeutic efficacy of primaquine in treating *Plasmodium vivax* acquired in southeast Asia and Oceania.

Keywords: Indonesia, *Plasmodium vivax* malaria, primaquine, relapse, Solomon Islands

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Imported *Plasmodium vivax* malaria continues to present diagnostic and therapeutic challenges in non-endemic regions [1–3]. *P. vivax* forms hypnozoites in the liver and requires primaquine therapy for radical cure. In recent years, primaquine-tolerant *P. vivax* strains have emerged in the Western Pacific, southeast Asia, South America and parts of Africa [2–4]. Primaquine-tolerant strains can relapse after the use of primaquine regimens involving a lower dosage or a shorter duration [3–5]. Currently, a primaquine regimen of 30 mg/day (paediatric dose, 0.5 mg/kg/day) for 14 days is recommended by the Centers for Disease Control and Prevention (Atlanta, USA) and the World Health Organization to eliminate the hypnozoites of vivax malaria acquired in Oceania and southeast Asia [5–7].

Taiwan successfully eliminated endemic malaria in 1965 and remains free from endemic malaria to date [8]. There are approximately 20–30 imported malaria cases annually. The Taiwan Centers for Diseases Control (CDC) (Taipei, Taiwan) have been notified of several cases of apparently recurring imported vivax malaria in patients who had received appropriate primaquine therapy. Some of these patients had travelled again to malaria-endemic areas for business after the apparently successful treatment of the first episode. We conducted a systematic investigation of all such cases occurring over the past 7 years, using molecular genotyping methods to assist in differentiating re-infection from true relapse.

From December 2003 to February 2010, there were a total of 52 confirmed cases of imported *P. vivax* malaria (including seven cases of mixed *P. vivax* and *P. falciparum* infection). The diagnosis of imported vivax malaria was established by the patients' travel history, microscopic examination and nested polymerase chain reaction (PCR) [9].

We reviewed the 52 cases and identified four patients with repeated episodes. All of these repeated episodes were caused by *P. vivax* alone. Blood samples were taken with each patient's consent according to the standard investigation procedure. All patient samples analysed were made anonymous. The study procedure was approved by the Taiwan CDC and the Institutional Review Board of National Taiwan University Hospital for exemption review.

The clinical and epidemiological information for the four cases was investigated. We defined low-dose primaquine regimen as 15 mg/day (paediatric dose, 0.25 mg/kg/day) for 14 days, standard-dose primaquine as 30 mg/day (paediatric dose, 0.5 mg/kg/day) for 14 days, and higher-dose primaquine as 60 mg/day (paediatric dose, 1 mg/kg/day) for 14 days.

The four patients are summarized in Table 1. The first episode was treated with standard-dose primaquine in patients 1, 2 and 3; low-dose primaquine was prescribed for patient 4. The second episodes occurred after an interval of

TABLE 1. Clinical and epidemiological data of four apparently recurrent vivax malaria cases

Patient	Age (years)/ Gender	Weight (BMI)	Inter-episode travel history	Area[a] of acquisition	Interval[b] (days)	Episode	Therapy
1	50/Male	67 kg (23.2)	Yes (80-day stay in Indonesia)	Indonesia	140	1.1	Chloroquine 500 mg/day for 2 days, then Primaquine 30 mg/day for 14 days[c]
						1.2	Chloroquine 500 mg/day for 2 days, then Primaquine 60 mg/day for 14 days[c]
2	52/Male	72 kg (23.5)	No	Solomon Islands	133	2.1	Chloroquine 500 mg/day for 2 days, then Primaquine 30 mg/day for 14 days[c]
						2.2	Chloroquine 500 mg/day for 2 days, then Primaquine 60 mg/day for 14 days[c]
3	38/Male	85 kg (24.8)	No	Indonesia	104	3.1	Chloroquine 500 mg/day for 2 days, then Primaquine 30 mg/day for 14 days[c]
						3.2	Chloroquine 500 mg/day for 2 days, then Primaquine 60 mg/day for 14 days[c]
4	46/Male	60 kg (20.8)	Yes (60-day stay in Thailand)	Thailand	469	4.1	Artesunate 2.4 mg/kg IV q12h for 3 doses, followed by 2.4 mg/kg IV qD for 3 days, then 100 mg/day for 3 days, then Primaquine 15 mg/day for 14 days[c]
						4.2	Artesunate 2.7 mg/kg IV q12h for 3 doses, followed by 2.7 mg/kg IV qD for 2 days, then 200 mg/day for 3 days, then Primaquine 30 mg/day for 14 days[c]

BMI, body-mass index = weight (kg)/(height (m))^2.
[a]Area where the patient acquired the first infection with imported vivax malaria.
[b]Interval: time between the first episode and the second episode.
[c]Administrated under direct supervision during hospitalization (median duration, 6 days) with patient education, followed by telephone monitoring after discharge until the completion of treatment.

3–16 months. The dosage of primaquine was doubled for the treatment of the second episode. Patients 1, 2 and 3 tolerated the higher-dose primaquine, and all of the patients remained free from both parasitaemia and further clinical recurrence with regular follow-up for up to 1 year.

Single nucleotide polymorphism analyses of the *P. vivax* dihydrofolate reductase gene [10,11] and PCR-restriction fragment length polymorphism analyses of the *P. vivax* merozoite surface protein 3β gene [12,13] were carried out using the previously described protocols [10,12]. The results (Table 2) indicated that the second episodes in patient 2, patient 3 and patient 4 represented relapses, while the second episode in patient 1 was possibly a re-infection.

Due to the long time intervals (3–16 months) between the first and second episodes, chloroquine or artesunate treatment failure can be excluded in these cases. The lack of travel history between episodes in patient 2 and patient 3 further supports the conclusion that their second episodes were relapses rather than re-infections. Notably, patient 2 and patient 3 (malaria acquired in the Solomon Islands and Indonesia, respectively) adhered to the standard-dose primaquine course and completed the treatment after the first episode. The body mass indices of the four patients (Table 1) are also in the normal range [14]. Therefore, the failure of standard-dose primaquine therapy to prevent relapse is not a result of underdosing in severely obese patients [15,16]. Relapse of vivax malaria despite completion of the recommended standard-dose primaquine regimen raises the possibility that the *in vivo* efficacy of primaquine as a treatment for *P. vivax* acquired in these regions may have decreased. Treatment with higher-dose primaquine for the second episode prevented further relapses in both patients. Higher-dose primaquine therapy, nonetheless, might increase the risk of methemoglobinaemia and serious haemolysis in patients with G6PD deficiency [17]. The experiences of these two patients highlight the need to continue monitoring of the therapeutic efficacy of primaquine in the treatment of *P. vivax* acquired in southeast Asia and Oceania. It is also crucial for both physicians and public health authorities to regularly follow-up returned travellers who acquired vivax malaria in these regions, after the completion of treatment.

The primaquine dosage administered for radical cure of the first episode in patient 4 (acquired in Thailand) was only half of that currently recommended [5–7]. The experience of patient 4 illustrates the risk of treatment failure associated with the old low-dose primaquine regimen for vivax malaria acquired in southeast Asia, including those cases acquired in Thailand.

Some reports found that parasites involved in relapse often bear a genotype different from those present at primary infection [18,19]. Thus, we cannot be certain that the

TABLE 2. Molecular genotyping analyses

Patient	Inter-episode travel history	Episode	*pvdhfr* polymorphism				Interpretation[a]
			F57I/L	S58R	T61M	S117 T/N	
1	Yes	1.1	L	R	M	T	Possible Re-infection
		1.2	L	S	M	T	
2	No	2.1	F/L	R	M/T	N/T	Relapse[b]
		2.2	L	R	T	T	
3	No	3.1	F	R	M	N	Relapse[c]
		3.2	F	R	M	N	
4	Yes	4.1	I	R	T	T	Relapse[c]
		4.2	I	R	T	T	

[a]Based on the combination of clinical and epidemiological data, *Plasmodium vivax* dihydrofolate reductase gene (*pvdhfr*) SNP analyses, and the *P. vivax* merozoite surface protein 3β gene (*pvmsp3*β) PCR-RFLP analyses.
[b]Relapse from one of the initial clones.
[c]The first and second episodes had identical *pvmsp3*β PCR-RFLP patterns.

second episode in patient 1 was definitely a re-infection. It could be the relapse of a hypnozoite strain other than that found in the initial parasitaemia. If this is indeed the case for Patient 1, then the clinical significance will be the same as that for patient 2 and patient 3.

In summary, this investigation identified two patients with imported vivax malaria, acquired in Indonesia and the Solomon Islands, respectively, who had a relapse after an interval of 3–4 months despite completing standard-dose primaquine therapy for treatment of the first episode. Treatment with higher-dose primaquine prevented further relapse. This finding calls for further monitoring of the therapeutic efficacy of primaquine in treating *P. vivax* acquired in southeast Asia and Oceania and for regular follow-up after the completion of treatment in individuals who acquired vivax malaria when travelling in these regions.

Transparency Declaration

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