

Short Report: Therapeutic Efficacy of Chloroquine in *Plasmodium vivax* and the *pvmdr1* Polymorphisms in the Republic of Korea Under Mass Chemoprophylaxis

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Abstract. Chemoprophylaxis with hydroxychloroquine (HCQ) and primaquine has been used in the Republic of Korea (ROK) Army since 1997. It may facilitate the development of chloroquine (CQ)-resistant strains of *Plasmodium vivax*. We investigated the therapeutic efficacy of HCQ and the *pvmdr1* gene polymorphisms in *P. vivax*. From June to September 2006, 102 soldiers with vivax malaria near the demilitarized zone in Gyeonggi-do, ROK, were enrolled in the study. We determined the status of compliance of chemoprophylaxis. In 85 patients, therapeutic efficacy was monitored 28 days after standard HCQ treatment; 66 (64.7%) of 102 malaria patients had taken all chemoprophylaxis with HCQ. In all patients enrolled in the therapeutic efficacy monitoring, parasitemia had not been observed since 3 days after standard HCQ treatment. However, the ubiquitous presence of the F1076L mutation of the *pvmdr1* was observed. There was no evidence that the F1076L mutation of *pvmdr1* could contribute to failure of HCQ treatment.

INTRODUCTION

By the end of 1970s, the World Health Organization (WHO) declared that *Plasmodium vivax* had been eradicated in the Republic of Korea (ROK).¹ In 1993, one case of malaria reemerged near the demilitarized zone (DMZ) that separates ROK (south) from the Democratic People's Republic of Korea (north).² Since 1993, the number of malaria cases has increased exponentially, not only among soldiers but also among civilians living near the DMZ.^{3,4} Although malaria did initially increase exponentially, the increase has since been tempered by control measures. Since 1997, the number of soldiers who received chemoprophylaxis with hydroxychloroquine (HCQ) and primaquine has continuously increased to over 200,000 in 2005. Nonetheless, the number of cases of malaria has increased again since 2005 in the ROK Army. Military antimalarial chemoprophylaxis has contributed, in part, to the decrease in the number of malaria cases among military personnel. However, long-term chemoprophylaxis may facilitate the development of chloroquine (CQ)-resistant strains of *P. vivax*.

Although the mechanism of *P. vivax* CQ resistance is unknown, it has been suggested that the mutation of *pvmdr1* that is the orthologue of the *pfmdr1* of *P. falciparum* is related to the CQ resistance.^{5,6}

We investigated the breakthrough malaria infection and the therapeutic efficacy of CQ in *P. vivax* in ROK. Additionally, the *pvmdr1* gene polymorphisms and their relevance to CQ resistance in *P. vivax* were evaluated.

MATERIALS AND METHODS

Study subjects. From June 1 to September 30, 2006, we investigated the malaria patients who were admitted to the three referral Armed Forces Hospitals, Byeokje, Yangju, and Ildong Hospitals, located in the Northern Kyunggi province near the DMZ. The patients were diagnosed when they had

any febrile illness and *P. vivax* in peripheral blood films. Blood samples were obtained from all patients who had given the informed consent. The protocol was approved by the Institutional Ethic Committee at the Armed Forces Medical Command (April 13, 2006).

Compliance of chemoprophylaxis. The chemoprophylaxis with HCQ sulfate (400 mg, one time per week) was started on May 8, 2006, and it continued throughout the transmission season (May to September). Therefore, the chemoprophylaxis was provided throughout the study period. Fourteen-day prophylaxis with primaquine (15 mg base, one time per day) was started on the first day of the last week of CQ administration.

The status of chemoprophylaxis was determined by the military record, which was a malaria case report form submitted by the military doctor after the interview with the patient. According to the status of chemoprophylaxis, the patients were classified as complete compliance when they took all doses before malaria infection, poor compliance when they did not take the dose more than one time before malaria infection, and no prophylaxis when they did not take the dose at all.

Monitoring of therapeutic efficacy. All patients diagnosed with vivax malaria received standard treatment: 2 g HCQ sulfate (day 1 dose, 1,200 mg; day 2 dose, 400 mg; day 3 dose, 400 mg) and 15 mg primaquine one time daily for 14 days. The patients were followed-up 28 days after the start of antimalarial treatment; the protocol used was a slightly modified version of that antimalarial drug resistance monitoring that was originally suggested by WHO (Table 1).⁷

Identification of *pvmdr1* polymorphism. Parasite genomic DNA from all blood samples collected into ethylenediaminetetraacetic acid (EDTA) tubes was extracted with a QIAamp DNA blood kit (QIAGEN Inc., Valencia, CA) following the manufacturer's instructions with minor modifications: the incubation time with proteinase K was increased to 1 hour at 56°C to improve the yield of the extraction, and DNA was eluted from the column by use of 100 µL polymerase chain reaction (PCR)-grade H₂O. Genomic DNA from *P. vivax* was amplified in a total volume of 20 µL containing 0.20 mmol/L 2'-deoxynucleoside 5'-triphosphate (dNTP), 1 µmol/L each primer, 1 U Platinum Taq DNA Polymerase (Invitrogen), 1.5 mmol/L MgCl₂, and 5 µL 10× PCR buffer.

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TABLE 1
Modified protocol of the WHO for antimalarial drug monitoring

Day	0	1	2	3	7	14	21	28
Clinical examination and temperature	×	×	×	×	×	×	×	×
Treatment with CQ	×	×	×					
Parasitemia	×			×	×	×	×	×
Molecular markers	×							

The sequences of PCR primers were as follows: *pvmdr1*-F (5'-CACCTTGCCCTTCTTCGT-3'), *pvmdr1*-R (5'-TTCACTTCTTGTTGCAAATAAC-3'), *pvmdr1*-S (5'-ATAGCATGCCCCAGGATTG-3'), and *pvmdr1*-AS (5'-ACGTTTGGTCTGGACAAGTATC-3'). PCR was performed under the following conditions: 37 cycles of denaturation at 94°C (5 min in the first cycle and 30 s in subsequent cycles), annealing for 30 s at 55°C, extension for 30 s at 72°C, and a final primer extension for 7 min. PCR products were sequenced using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences were compared with the *pvmdr1* (GenBank accession number AY618622) of Sal-1, a CQ-susceptible strain from Salvador,⁸ using a basic local alignment search tool (BLAST) search of the National Center for Biotechnology Information databases (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

RESULTS

During the study period, a total of 149 patients were diagnosed as *P. vivax* malaria in the study hospitals. The status of chemoprophylaxis was determined for 102 patients whose military records were available, and the therapeutic efficacy was monitored for 28 days after standard HCQ treatment in the 85 patients who had given the informed consent (Figure 1); 66 (64.7%) patients were classified as complete compliance, 11 (10.8%) patients had poor compliance, and 25 (24.5%) patients had no prophylaxis (Table 2). All peripheral blood smears needed for monitoring had been done in all 85 patients, and all enrolled patients cleared their parasites within 3 days.

The PCR was performed from the blood samples that were obtained from the 85 patients who enrolled in therapeutic efficacy monitoring. After PCR, the 78 isolates were obtained successfully from the 85 patients. After sequencing the PCR products, the single-nucleotide polymorphisms of *pvmdr1* were analyzed, and the phenylalanine (F) of codon 1076 in *pvmdr1* was found to be substituted by leucine (L) in all isolates, including 63 patients with complete compliance. The other mutations, including Y976F, were not found.

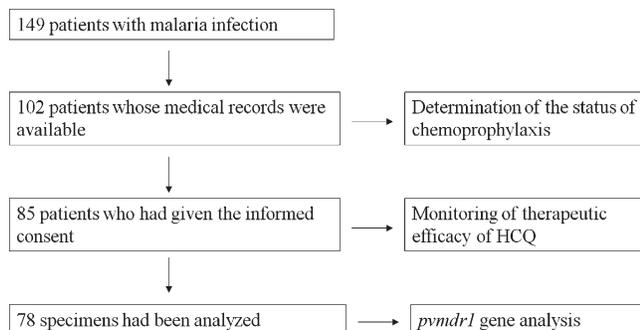


FIGURE 1. Study enrollment flowchart.

TABLE 2
Baseline characteristics of patients with vivax malaria

Characteristics* (N = 102)	Measurement
Mean age (in years) ± standard deviation (range)	22.5 ± 3.3 (17–36)
Residency	
Northwestern, Kyeonggi (Byeokje)	44 (43.1%)
Mid-north, Kyeonggi (Yangju)	36 (35.3%)
Northeastern, Kyeonggi (Ildong)	23 (22.6%)
Compliance	
Complete	66 (64.7%)
Poor	11 (10.8%)
No	25 (24.5%)
Median parasitemia count per microliter (IQR†)	2,250 (490–8050)‡

*All patients were male.

†IQR = interquartile range.

‡Complete compliance group (2,057/1 μL) vs. poor and no prophylaxis groups (4,558/1 μL).

DISCUSSION

The mass chemoprophylaxis for the soldiers based in the risk area reduced the number of malaria cases, but long-term chemoprophylaxis could facilitate the development of drug resistance/tolerance or prophylaxis failure.^{9,10} In this study, 66 patients were defined as complete compliance by the military record. The drug level in whole blood of the patients with chemoprophylaxis was not measured at the malaria infection; it is highly likely that the drug concentration in patients with complete compliance could be higher than 100 ng/mL, because it is usually known that the drug concentration is maintained higher than 100 ng/mL by the long-term CQ prophylaxis given one time per week.¹¹ *P. vivax* may be classified as biologically resistant if malaria infection that persists in the CQ level in whole blood is greater than 100 ng/mL, and it should completely cure blood-stage CQ-sensitive *P. vivax*.¹² However, Lee and others¹³ showed that the HCQ and its metabolite could not be found in the blood of 46 of 74 soldiers who had taken HCQ chemoprophylaxis weekly, although the blood concentration of the HCQ and its metabolites was above 100 ng/mL in eight patients. Therefore, it is not truly breakthrough malaria in the 66 patients regarded as complete compliance group.

The *pfmdr1* gene of *P. falciparum* is classified as the adenosine 5'-triphosphate (ATP) binding cassette (ABC) transporter, and it has been known to relate with the resistance against CQ, artemisinin, quinine, mefloquine, and halofantrine.¹⁴ The *pvmdr1* gene of *P. vivax* is the orthologue of the *pfmdr1* of *P. falciparum*, and there is controversy regarding whether the *pvmdr1* gene relates with the CQ resistance.^{5,6,15,16} A recent study showed that the amplification of *pvmdr1* and single-nucleotide polymorphisms was correlated with susceptibility of *P. vivax* to multiple antimalarial drugs and that the *pvmdr1* Y976F allele had reduced susceptibility to CQ.¹⁷ In this study, the F → L change at codon 1076 of the *pvmdr1*, which was caused by a single mutation (TTT → CTT), was observed in all 78 isolates. Therefore, this ubiquitous presence of the F1076L mutation of the *pvmdr1* might be correlated with geographical characteristics in Korean vivax malaria rather than drug resistance.

In this study, no treatment failure was observed after the standard treatment of CQ/primaquine in 85 patients for the 28 days of monitoring. Considering the blood schizonticidal effect of primaquine,¹⁸ it could not be determined that there was absolutely no CQ treatment failure, but it could be determined that there was no standard treatment failure.

In this study, there were limitations. For example, the blood levels of CQ and its metabolites, desethylchloroquine and desethylhydroxychloroquine, were not measured after the vivax malaria was developed. In conclusion, there was no treatment failure of HCO and primaquine in Korea, and there was no evidence that the F1076L mutation of *pvmdr1* could contribute to HCO resistance in *P. vivax*.

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REFERENCES

- World Health Organization, 1981. Synopsis of the world malaria situation in 1979. *Wkly Epidemiol Rec* 56: 145–149.
- Chai IH, Lim GI, Yoon SN, Oh WI, Kim SJ, Chai JY, 1994. Occurrence of tertian malaria in a male patient who has never been abroad. *Korean J Parasitol* 32: 195–200.
- Park JW, Klein TA, Lee HC, Pacha LA, Ryu SH, Yeom JS, Moon SH, Kim TS, Chai JY, Oh MD, Choe KW, 2003. Vivax malaria: a continuing health threat to the Republic of Korea. *Am J Trop Med Hyg* 69: 159–167.
- Yeom JS, Ryu SH, Oh S, Lee WJ, Kim TS, Kim KH, Kim YA, Ahn SY, Cha JE, Park JW, 2005. Status of *Plasmodium vivax* malaria in the Republic of Korea during 2001–2003. *Am J Trop Med Hyg* 73: 604–608.
- Brega S, Meslin B, de Monbrison F, Severini C, Gradoni L, Udomsangpetch R, Sutanto I, Peyron F, Picot S, 2005. Identification of the *Plasmodium vivax* mdr-like gene (*pvmdr1*) and analysis of single-nucleotide polymorphisms among isolates from different areas of endemicity. *J Infect Dis* 191: 272–277.
- Suwanarusk R, Russell B, Chavchich M, Chalfein F, Kenangalem E, Kosaisavee V, Prasetyorini B, Piera KA, Barends M, Brockman A, Lek-Uthai U, Anstey NM, Tjitra E, Nosten F, Cheng Q, Price RN, 2007. Chloroquine resistant *Plasmodium vivax*: *in vitro* characterisation and association with molecular polymorphisms. *PLoS One* 31: e1089.
- World Health Organization, 2003. *Monitoring Antimalarial Drug Resistance: A Report of a Consultation*. Geneva, Switzerland: World Health Organization.
- Contacos PG, Collins WE, Jeffery GM, Krotoski WA, Howard WA, 1972. Studies on the characterization of *Plasmodium vivax* strains from Central America. *Am J Trop Med Hyg* 21: 707–712.
- Yeom JS, Ryu SH, Oh S, Choi DH, Song KJ, Oh YH, Lee JH, Kim YA, Ahn SY, Yang HY, Cha JE, Park JW, 2005. Evaluation of anti-malarial effects of mass chemoprophylaxis in the Republic of Korea army. *J Korean Med Sci* 20: 707–712.
- Park K, Kang JK, 2003. Effectiveness of primaquine terminal prophylaxis against late primary attacks of *Plasmodium vivax* malaria: a case-control study among troops of the Republic of Korea army. *Trans R Soc Trop Med Hyg* 97: 441–442.
- Rombo L, Berqvist Y, Hellgren U, 1987. Chloroquine and desethylchloroquine concentrations during regular long-term malaria prophylaxis. *Bull World Health Organ* 65: 879–883.
- Baird JK, 2004. Chloroquine resistance in *Plasmodium vivax*. *Antimicrob Agents Chemother* 48: 4074–4083.
- Lee SW, Lee M, Lee DD, Kim C, Kim YJ, Kim JY, Green MD, Klein TA, Kim HC, Nettey H, Ko DH, Kim H, Park I, 2009. Biological resistance of hydroxychloroquine for *Plasmodium vivax* malaria in the Republic of Korea. *Am J Trop Med Hyg* 81: 600–604.
- Peel SA, 2001. The ABC transporter genes of *Plasmodium falciparum* and drug resistance. *Drug Resist Updat* 4: 66–74.
- Picot S, Brega S, G er ome P, Velut G, de Monbrison F, Cheminel V, Peyron F, 2005. Absence of nucleotide polymorphism in a *Plasmodium vivax* multidrug resistance gene after failure of mefloquine prophylaxis in French Guyana. *Trans R Soc Trop Med Hyg* 99: 234–237.
- S a JM, Nomura T, Neves J, Baird JK, Wellems TE, del Portillo HA, 2005. *Plasmodium vivax*: allele variants of the mdr1 gene do not associate with chloroquine resistance among isolates from Brazil, Papua, and monkey-adapted strains. *Exp Parasitol* 109: 256–259.
- Suwanarusk R, Chavchich M, Russell B, Jaidee A, Chalfein F, Barends M, Prasetyorini B, Kenangalem E, Piera KA, Lek-Uthai U, Anstey NM, Tjitra E, Nosten F, Cheng Q, Price RN, 2008. Amplification of *pvmdr1* associated with multidrug-resistant *Plasmodium vivax*. *J Infect Dis* 198: 1558–1564.
- Baird JK, Hoffman SL, 2004. Primaquine therapy for malaria. *Clin Infect Dis* 39: 1336–1345.