

Malaria Parasites Detected in Myanmar Thalassemia Patients

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ABSTRACT. We examined the existence of malaria parasite carriers in 186 Myanmar transfusion dependent anemic patients with thalassemia mutations. Two malaria parasites, *Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*), were detected by the PCR method using a multiplex primer set. Six patients were infected with *Pf*, and seven with *Pv*. The β -thalassemia (thal) genotypes and hemoglobinopathies were as follows; combination homozygote and/or heterozygote with Hb E and β^0 -thal or β^+ -thal, β^0 -thal and β^0 -thal, or β^0 -thal and β^+ -thal. Among these patients, one had Hb H disease, one α -thal-1 of the Southeast Asian genotype, and one a combination heterozygote of β^0 -thal with α -thal-2. Although they were *Pf* or *Pv* carriers, they manifested no clinical symptoms due to malaria infection. Therefore, it might be suggested that thal patients have resistance to malaria.

Key words: Malaria parasites — β -Thalassemia — α -Thalassemia —
Polymerase chain reaction (PCR) — Myanmar

In our previous paper, we reported that the incidence of carriers of the malaria parasites, *Plasmodium falciparum* (*Pf*) and/or *P. vivax* (*Pv*) among the healthy Myanmar population was small, being 2.3%.¹⁾ In the present study, the number of carriers of *Pf* and/or *Pv* among transfusion dependent anemic patients with some thalassemia (thal) mutations was investigated.

MATERIALS AND METHODS

The subjects of this investigation were 186 transfusion dependent anemic patients who visited the Day Care Room of the Outpatient Department of Yangon Children's Hospital from various Myanmar states (Bamar, Kayin, Mon, Rankhine, Shan and Chin) and who were diagnosed as patients having combination homozygote or heterozygote Hb E with thal mutation.^{2,3)} Red cells separated from the plasma of their peripheral blood and frozen for transportation were used as blood samples.

First, analyses of hemolysates were done by anion exchange resin high

performance liquid chromatography (DEAE-HPLC) and isoelectric focusing (IEF) to identify Hb E carriers.^{2,3)} DNA extracted from their red cells by a simple method using a Qiagen DNA Extraction Kit was amplified with a specific primer set for the detection of *Pf* and/or *Pv*. Amplified DNA was electrophoresed on 2% Nusieve gel and a picture was taken under ultraviolet light after staining with ethidium bromide. A specific primer set was used to confirm the respective parasites. The appearance of a 206 bp DNA fragment band indicated the presence of *Pf* and of a 121 bp band the presence of *Pv*.^{4,5)}

RESULTS

Transfusion dependent patients investigated here showed no clinical symptoms of malaria such as fever. Thirteen patients were identified as carriers of *Pf* and/or *Pv*. Further investigation showed six patients to be *Pf* carriers and seven to be *Pv* carriers (Fig 1). Ten patients, excluding My-H3 with Hb H disease and My-H46 with α -thal-1, were combinations homozygote or heterozygote Hb E with a β^0 -thal mutation or a β^+ -thal mutation (Table 1). However, the mutation of one patient (My-74) could not be identified, although gene analysis of his β -globin gene showed to be heterozygous.

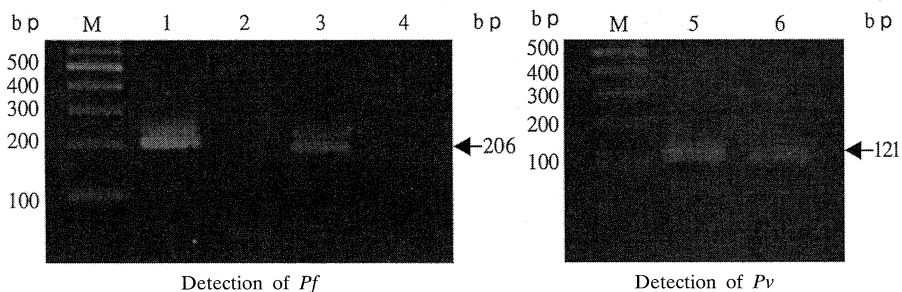


Fig 1. The detection of malaria parasites, *Pf* or *Pv*, in thalassemia patients by electrophoresis of the DNA products amplified using a specific primer set on 2% Nusieve after staining with ethidium bromide. M: Molecular marker. 1: Control with *Pf*. 2 and 4: My-191 and My 14 (negative case for *Pf*). 3: My-7 (positive case for *Pf*). 5: Control with *Pv*. 6: My-109 (positive case for *Pv*).

DISCUSSION

The patients visited the Day Care Room of the Outpatient Department of the Yangon Children's Hospital from various states in Myanmar. Some came from mountainous areas to undergo blood transfusions for treatment of anemia. Blood was collected from the patients between May and July; i.e., from the end of the dry season to the beginning of the rainy season. The breeding of malaria mosquitos occurs most commonly during the rainy season and in mountainous areas. We could not completely clarify circumstances in the states where the patients lived and the time when they were infected with malaria parasites. They are patients requiring regular or irregular blood transfusions for the treatment of anemia. The incidence of

TABLE 1. *Pf* and/or *Pv* in Myanmar thalassemia patients detected by the PCR and results analyzed by DEAE-HPLC and IEF.

Patient No.	Hb A ₂ +Hb E (%)	Hb E* ¹ (+ or -)	<i>Pf</i> or <i>Pv</i>	Thal genotypes
My-H3	3.8	(-)	<i>Pv</i>	- ^{SEA} /- $\alpha^{3,7}$: Hb H Disease
My-H6	37.0	(+)	<i>Pv</i>	Hb E/ β IVS I-5 G→C: Hb E+ β^+ -thal
My-H36	55.0	(+)	<i>Pf</i>	Hb E/Hb E: Hb E homozygote
My-H46	1.8	(-)	<i>Pv</i>	- ^{SEA} / $\alpha\alpha$: α -thal-1
My-4	4.4	(-)	<i>Pf</i>	CD41-42 TTCTTT→TT/ β IVS I-1 G→T : β^0 -thal compound heterozygote
My-7	1.8	(-)	<i>Pf</i>	β IVS I-1 G→T/ β IVS I-1 G→T/- $\alpha^{3,7}$ / $\alpha\alpha$: β^0 -thal homozygote+ α -thal-2
My-15	67.0	(+)	<i>Pf</i>	Hb E/CD17 A→T: Hb E+ β^0 -thal
My-53	3.8	(-)	<i>Pv</i>	β IVS I-5 G→C/ β IVS I-5 G→C : β^+ -thal homozygote
My-72	1.7	(-)	<i>Pf</i>	β IVS I-1 G→T/ β IVS I-1 G→T : β^0 -thal homozygote
My-74	3.6	(-)	<i>Pf</i>	ND but heterozygote for β -globin gene
My-78	3.3	(-)	<i>Pv</i>	CD41-42 TTCTTT→TT/ β IVS I-1 G→T : β^0 -thal compound heterozygote
My-109	18.0	(+)	<i>Pv</i>	Hb E/ β IVS I-1 G→T: Hb E+ β^0 -thal
My-181	33.0	(+)	<i>Pv</i>	Hb E/ β IVS I-1 G→T: Hb E+ β^0 -thal

*¹The Hb A₂ plus Hb E level (%) was estimated by DEAE-HPLC and the presence of Hb E was determined by DEAE-HPLC, IEF, and DNA analysis.

malaria parasite carriers was 7% in total, and 3.2% and 3.8% for *Pf* and *Pv*, respectively. This was higher than that of the healthy Myanmar population reported in the previous paper (2.3% in total).¹⁾ Generally, people having either thal syndrome or hemoglobinopathy; e.g. Hb E, have been considered to have resistance to infection with malaria parasites.^{6,7)} None of the malaria carriers among the thal patients investigated here showed any clinical symptoms of malaria such as fever.

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