

Hemoglobinopathies and Malaria Infection in Myanmar

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ABSTRACT. Screening for the incidences of hemoglobinopathies (Hb E, α -thalassemia (thal) and β -thal) and malaria infection was done in 131 healthy volunteers with no clinical symptoms in Shan State in Myanmar. The group consisted of 71 females and 60 males ranging in age from 16 to 46 years old. The analysis of hemoglobin was carried out using high performance liquid chromatography (HPLC) and isoelectric focusing (IEF), and DNA analysis was done by polymerase chain reaction (PCR)-electrophoresis. The incidences of carriers of Hb E, α -thal and β -thal were 17.6%, 7.6% and 0.8%, respectively. The thal genotypes observed were $-\alpha^{3,7}$, which is an α -thal-2 mutation, and CD17A \rightarrow T, which is a β^0 -thal mutation. Both are common mutations in Myanmar. There were only three (2.3%) carriers of two malaria parasites, *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv); namely one female carrier of Pf and two male carriers of Pv. Although it was suspected that the incidence of subjects infected with malaria would be high, there was comparatively small number of cases. This may be the reason why the blood sample collection from the volunteers was done during the dry season which is not the breeding season for malaria mosquitos.

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

Myanmar is a South Asian country in which hemoglobinopathies [abnormal hemoglobin (Hb), especially Hb E, and α - and β -thalassemias (thal)] and malaria parasites are prevalent.¹⁾ In our previous papers concerning molecular diagnosis of transfusion-dependent anemic Myanmar patients, we reported that high incidences of carriers of Hb E and various kinds of α - and β -thals were observed.²⁻⁴⁾ Eighteen types of β -thal mutations, including four main mutations (codon (CD)17 A \rightarrow T, β -intervening sequence (β IVS) I-1 G \rightarrow T, β IVS I-5 G \rightarrow C and CD41/42 TTCTTT \rightarrow TT) and two deletion types of α -thal mutations (the $-\alpha^{SEA}$ genotype of α -thal-1 and the $-\alpha^{3,7}$ genotype of α -thal-2) were detected. We also described the

methodology for highly sensitive detection of *Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*), and its application to abnormal Hb patients of the Solomon Islands.⁵⁾

At this time, we had the opportunity to screen for carriers of hemoglobinopathies (abnormal Hb E, α - and/or β -thals) and malaria parasites (*Pf* and/or *Pv*) among inhabitants of Shan State in Myanmar.

MATERIALS AND METHODS

Blood samples from 131 healthy volunteers, 71 females (JF series) and 60 males (JM series), ranging in age from 16 to 46 years old living in Shan State in Myanmar were collected in tubes with EDTA-2Na as an anticoagulant in the period from the middle of February through the end of March, 2002. Red cells, which had been separated from plasma by means of centrifugation, were brought to the Department of Biochemistry, Kawasaki Medical School, Kurashiki, Japan. The hemolysates prepared from 200 μ L of red cells and 400 μ L of distilled water were analyzed using anion exchange high performance liquid chromatography (DEAE-HPLC) on a DEAE-5PW column (7.5 \times 75 mm, Tosoh Corporation, Tokyo, Japan) to detect the presence of abnormal Hbs; e.g., Hb E, and to determine the proportions of Hb components (Hb A₂, Hb A, Hb F and any additional abnormal Hb).²⁻⁴⁾ Isoelectric focusing (IEF, pH range: 6-9) was also used for the analysis of Hb components. DNA was extracted from the red cells by a simple method using a Qiagen DNA Extraction Kit and the method of Poncz *et al*⁶⁾ with slight modification. Detection of *Pf* and *Pv* was done using the same procedure, namely the multiplex PCR method, as described previously.⁵⁾

Confirmation of Hb E was done employing the same procedures as described previously; i.e., amplified DNA digested with the restriction enzyme *Mnl* I was electrophoresed on 5% polyacrylamide gel and silver stained, and the appearance of a DNA fragment of 231 bp signified presence of the β^E -globin gene.^{2,7)} α -Thal mutations, especially the α -thal-2 ($-\alpha^{3,7}$) and α -thal-1 mutations ($--^{SEA}$ or $--^{Med}$), were detected in all subjects by the multiplex PCR method.^{4,8)} DNA from subjects with relatively high values of Hb A₂ from 2.8 to 5.32% was analyzed for the common Myanmar β -thal mutations (CD17 A \rightarrow T, β IVS I-1 G \rightarrow T, β IVS I-5 G \rightarrow C and CD41/42 TTCTTT \rightarrow TT) using the amplification refractory mutation system (ARMS) described previously.³⁾

RESULTS

The chromatographic (HPLC) and electrophoretic (IEF) behaviors of Hb E are similar to those of Hb A₂ (Fig 1). The distributions of the values obtained by DEAE-HPLC analysis for Hb A₂ ranged from 1.0 to 5.5%, while that for Hb E plus Hb A₂ ranged from 17.5 to 35%. These data are shown in Fig 2, where the mean values for Hb A₂ and Hb E plus Hb A₂ were 2.17% and 26.0%, respectively, and the standard deviations were 0.49 and 4.70. The lower value of the Hb E plus Hb A₂ than the normal (generally the stable abnormal Hb content of a subject heterozygous for the

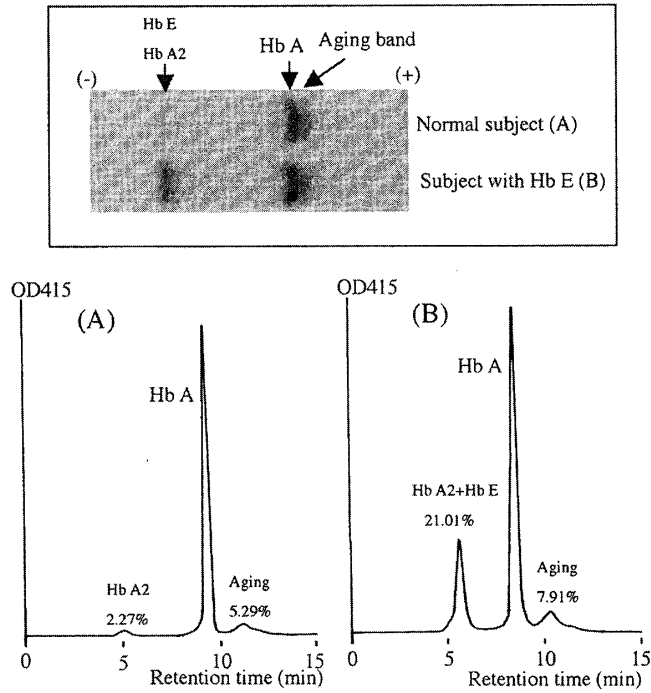


Fig 1. IEF patterns and chromatograms of DEAE-HPLC obtained from hemolysates. A : Normal subject. B : Subject with Hb E.

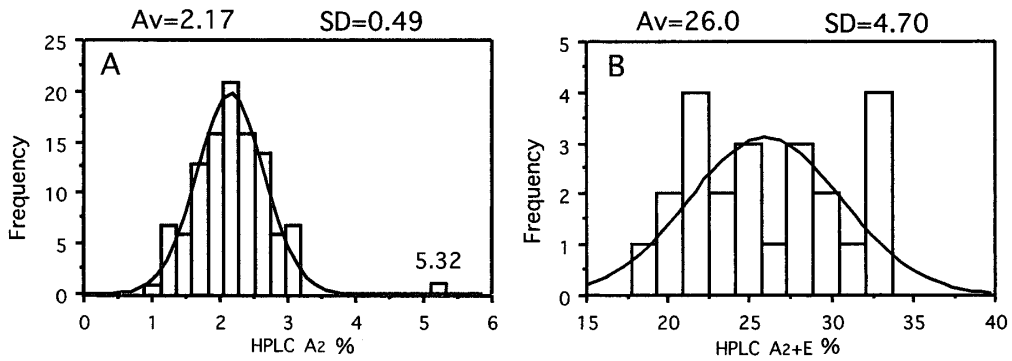


Fig 2. Histograms of subjects having Hb A₂ values of less than 5.5% (A) and with Hb E plus Hb A₂ values between 15% and 35% (B). Av : Mean value. SD : Standard deviation.

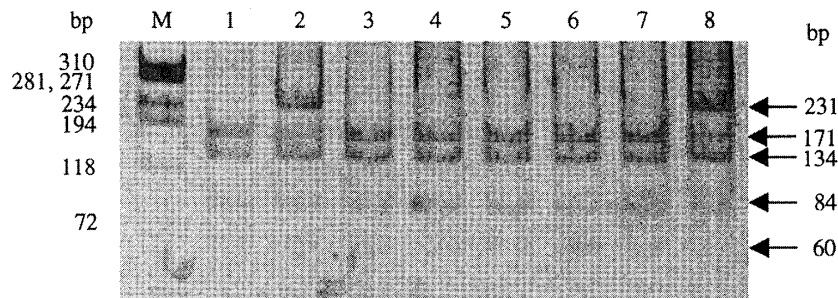


Fig 3. Detection of β^E -globin gene by electrophoresis of the PCR product digested with *Mnl* I on 5% polyacrylamide gel and silver staining. M : Molecular marker. 1 and 3-7 : JM-32 and JM-34-38 (normal cases). 2 and 8 : JM-33 and JM-39 (heterozygous cases for β^E -globin gene).

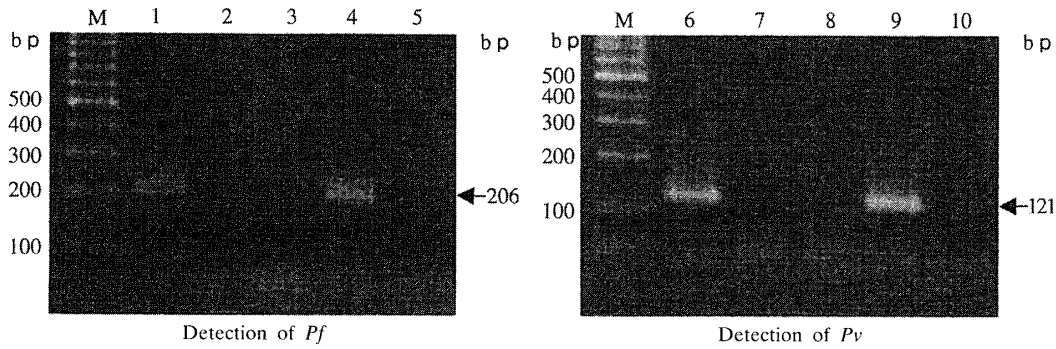


Fig 4. Detection of the malaria parasites, *Pf* or *Pv*, by electrophoresis of the product amplified by the multiplex PCR method on 2% Nusieve gel. M: Molecular marker. 1: control with *Pf*. 2, 3 and 5: JF-1, JF-2 and JF-70 (negative cases of *Pf*). 4: JF-69 (positive case for *Pf*). 6: Control with *Pv*. 7, 8 and 10: JM-11, JM-12 and JM-14 (negative cases for *Pv*). 9: JM-13 (positive case for *Pv*).

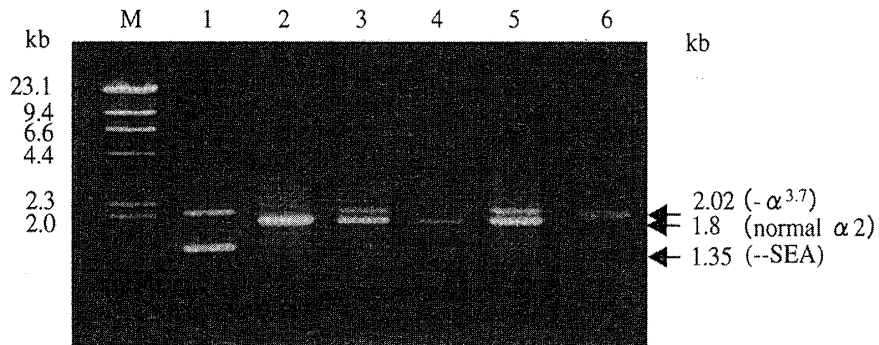


Fig 5. Detection of the $-\alpha^{3.7}$ genotype of the α -thal-2 mutation by electrophoresis of the DNA product amplified by multiplex primer sets on 1% agarose gel. M: Molecular marker. 1: Control with genotype $-\alpha^{3.7}/-^{SEA}$. 2 and 4: JF-1 and JF-3 (normal cases). 3 and 5: JF-2 and JF-4 (cases with genotype $-\alpha^{3.7}/\alpha\alpha$). 6: JF-13 (case with genotype $-\alpha^{3.7}/-\alpha^{3.7}$).

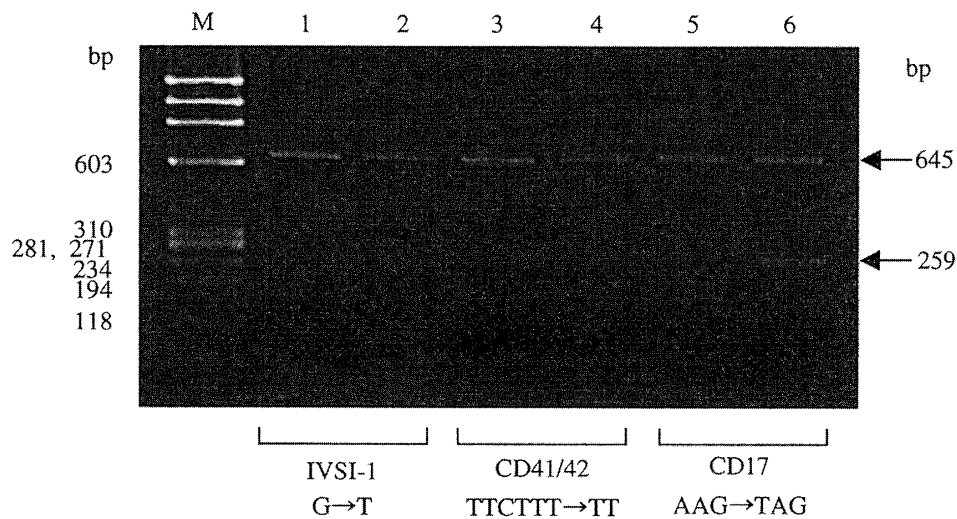


Fig 6. Detection of the CD17 A \rightarrow T β^0 -thal mutation by electrophoresis of the DNA produced by the ARMS method. M: Molecular marker. 1, 3 and 5: JM-5. 2, 4 and 6: JM-6.

β -globin chain anomaly is estimated to be approximately 45%) is suspected to have been caused by a β^E -globin gene with a β -thalassemic property.⁹⁾ The Hb A₂ value of one subject (JM-6) was 5.32%, when it led us to suspect that the subject might have a β -thal mutation. These results were comparable to the IEF findings. There were 23 Hb E heterozygote carriers; 11 females and 12 males, estimated to be 17.6% of the total subjects.

The DNA of 23 subjects with a high Hb A₂ value was amplified and electrophoresed after digestion with *Mnl* I. Combination of the 171 bp band with the 60 bp band resulting in a 231 bp band with loss of the restriction site of *Mnl* I was found in 23 subjects (Fig 3), indicating to be the Hb E carriers.

Pf and/or *Pv* were detected with a multiplex primer set or a specific primer set in three subjects (about 2.3%). *Pv* was found in two males (JM-2 and JM-13), and *Pf* in one female (JF-69). These findings were reconfirmed using a specific primer set to detect individual *Plasmodium* (Fig 4).

We detected α -thal in 10 subjects (JM-14, JM-20, JM-56, JM-60, JF-2, JF-4, JF-13, JF-26, JF-62, and JF-70) by the PCR method using multiplex primer sets. The genotypes found in them were all $-\alpha^{3,7}$ of the α -thal-2 mutation. No genotypes $--^{SEA}$ and $--^{MED}$ of the α -thal-1 mutation were found (Fig 5). Except for JM-56, they had a relatively lower level of Hb A₂. Among them, JF-13 was homozygous for genotype $-\alpha^{3,7}$. JM-20 and JM-60 were also carriers of Hb E, being compound heterozygotes of Hb E and α -thal-2, $-\alpha^{3,7}$ genotype. The total percentage of α -thal carriers was about 7.6%. The Hb A₂ values of the carriers of α -thal ranged between 1.2 and 2.4%, which were relatively lower values.

TABLE 1. Results of the detection of Hb E, α - or β -thal mutation, and/or malaria parasite, *Pf* or *Pv*.^{*1}

Subjects	Hb A ₂ +Hb E (%)	Hb E (+ or -)	<i>Pf</i> or <i>Pv</i>	Thal mutations	
				α -thal	β -thal
JM-2	2.5	(-)	<i>Pv</i>	(-)	(-)
-6	5.3	(-)	(-)	(-)	CD17 A→T
-13	2.6	(-)	<i>Pv</i>	(-)	(-)
-14	2.3	(-)	(-)	$-\alpha^{3,7}$	(-)
-20	32.5	(+)	(-)	$-\alpha^{3,7}$	(-)
-56	3.0	(-)	(-)	$-\alpha^{3,7}$	(-)
-60	20.6	(+)	(-)	$-\alpha^{3,7}$	(-)
JF-2	2.4	(-)	(-)	$-\alpha^{3,7}$	(-)
-4	2.2	(-)	(-)	$-\alpha^{3,7}$	(-)
-13	1.6	(-)	(-)	$-\alpha^{3,7}$ (homo-)	(-)
-26	1.3	(-)	(-)	$-\alpha^{3,7}$	(-)
-62	2.2	(-)	(-)	$-\alpha^{3,7}$	(-)
-69	1.2	(-)	<i>Pf</i>	(-)	(-)
-70	1.2	(-)	(-)	$-\alpha^{3,7}$	(-)

^{*1}Carriers of the Hb E heterozygote were excluded from this Table. Percentages of Hb A₂ plus Hb E were calculated from DEAE-HPL chromatograms.

In JM-6 and the subjects having higher Hb A₂ values than 2.8%, four common β -thal mutations in Myanmar (CD17 A→T, β IVS I-1 G→T, β IVS I-5 G→C and CD41/42 TTCTTT→TT) were detected by the ARMS. As suspected, JM-6 had a β^0 -thal mutation of CD17 A→T (Fig 6). In this study, therefore, the rate of β -thal carriers was 0.8% in total. Among the carriers of Hb E and/or thal mutations, none was infected with *Pf* or *Pv*. The data are summarized in Table 1.

DISCUSSION

Myanmar is considered as an Asian country with a prevalence of malaria parasites and hemoglobinopathies (Hb E, α -thal and β -thal).¹⁾ The incidence of both in rural and mountainous regions tends to be higher than in urban areas. In this study, peripheral blood samples from 131 healthy volunteers with no clinical symptoms who were in Shan State, a mountainous region, were examined. The blood samples were collected in the period from the middle of February through the end of March, which is the dry season in Myanmar, and, therefore, not the breeding season for malaria mosquitos.

In this study, although 17.6% of the volunteers were Hb E carriers, the numbers of α - and β -thal (7.6% and 0.8%, respectively) and malaria parasite carriers (2.3%) (Table 1), were comparatively fewer than expected before this study. However, as expected, there was a high frequency of the $-\alpha^{3.7}$ genotype of the α -thal-2 mutation.^{4,10)} Unexpectedly, for the β -thal genotype, only a CD17 A→T of β^0 -thal mutation was encountered, although it is one of the most common Myanmar β -thal.^{3,10)} Additionally, only 2.3% of the subjects were carriers of the malaria parasite, *Pf* and/or *Pv*. No carriers with both hemoglobinopathy and malaria parasite were detected in the subjects, but some hemoglobinopathies (Hb E, α -thal or β -thal) were detected in 32 subjects without *Pf* or *Pv* infection. The remaining 99 subjects (75.6%) were free of hemoglobinopathies and malaria parasites. This might have been the reason why they were healthy and had no clinical symptoms. Although it has been generally supposed that carriers of hemoglobinopathies, Hb E or thal, are resistant to malaria parasites, this study could not make clarify that supposition.

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