Prevalence of Hemoglobin E Among the Children Taking Regular Blood Transfusion at the Day Care Room, Yangon Children Hospital, Myanmar

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Accepted for publication on February 17, 2001

ABSTRACT. Peripheral blood of 132 children (73 boys and 59 girls; nine months to 12 year old; Bamar, Kayin, Mon, Rakhine, Shan, Chin ethnic groups), who had been clinically diagnosed as thalassemia major and were taking frequent and regular blood transfusion at the day care room, Yangon Children Hospital, Myanmar. Before blood transfusion, blood sample was collected in EDTA as an anticoagulant and examined red cell morphology and total Hb level for the diagnosis of anemia. The hemolysate was prepared from the red cells and subjected for anion-exchange high perfomance liquid chromatography (DEAE-HPLC) and isoelectric focusing (IEF) to detect and analyze different Hb present. Eighty four out of 132 samples (63.6%) showed large peak in DEAE-HPLC and thick clear band in IEF representing the presence of Hb E. Being not separated, Hb A_2 was also included in the concentration of Hb E calculated from chromatogram. The presence of Hb E was confirmed by β^E globin gene detection of a restriction enzyme (Mnl I) assay of PCR products obtained from the genomic DNA. Since Hb E prevalence was found to be very high in this study, accurate determination of Hb A_2 was important for the carrier screening in Myanmar thalassemia and more importantly Hb E should be included as the most common mutant in a protocol for an effective prenatal diagnosis program in Myanmar.

Key words: Hemoglobin E (Hb E) — isoelectric focusing (IEF) — DEAE-high performance liquid chromatography (DEAE-HPLC) — polymerase chain reaction (PCR) — Myanmar

Myanmar is situated in Southeast Asia region where thalassemias (Thal) of different forms are highly prevalent and hemoblobin E (Hb E) is the most common abnormal Hb in this region. The prevalence of Hb E in the general population of Myanmar has been approximated to be 25%.[1)]

The Outpatient Department of Yangon Children Hospital (YCH) has a day care room (DCR) which was established since May 1982 for patients requiring regular blood transfusion. The YCH is the largest hospital for the children in Myanmar and is also a referral hospital which covers the

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population both from Yangon and from other parts of the country.

Currently, Myanmar and Japanese scientists have a joint effort to study Myanmar Thal/hemoglobinopathies from many aspects such as iron overload and its effect on the hepatocarcinogenesis via free radicals effect,[2] chronic hepatitis viral markers studies,[3] globin gene mutation analysis, etc. in the patients attending at the DCR, YCH, for chronically severe and refractory anemia, with a clinical diagnosis of Thal or hemoglobinopathy. According to the DCR registry, more than 93% of cases are diagnosed as thalassemias.[4]

A total of 132 cases involved in this study. Patients of under 12 year children (nine months to 12 year), both sex (73 boys and 59 girls) and of any race or ethnic group (Bamar, Kayin, Mon, Rakhine, Shan, Chin) attending at DCR for blood transfusion were included. After getting an informed consent, blood samples were collected with EDTA as the anticoagulant, separated into plasma, buffy coat and red cell pellet at Pathology Research Division, Department of Medical Research (Lower Myanmar), Yangon, and were transported in dry ice from Yangon to Japan for further investigations. Relevant clinical data were taken from the patient's register and record.

After the preparation of hemolysate from the red cell pellet of all samples, DEAE-HPLC[5] and isoelectric focusing[6] were done. The relative proportion of Hb E, Hb A_2, Hb A, and Hb F of the total Hb content was calculated from the chromatogram on area basis and shown in percentage. DNA was extracted from the blood cells by using QIAmp DNA Mini Kit[7] and restriction enzyme analysis of the PCR products by use of proper PCR primer sets was done for the confirmation of presence of Hb A and/or Hb E genes as described previously.[8]

We observed HPL-chromatogram together with IEF plate to make a decision of hemoglobin phenotypes. Hb peak observed between 5 min. and 6 min. of retention time of HPLC was identified as either Hb A_2 or Hb E or mixed (Fig 1). That peak was observed in all of the samples. Since Hb E and Hb A_2 were not separated in the chromatogram, area concentration represents for both Hb. The concentration ranged from 0.9% to 88.9%. Therefore, it was a practical problem to get the actual concentration of either Hb E or Hb A_2 from the chromatogram.

We tried with another program of DEAE-HPLC for a better separation of Hb E and Hb A_2 (Fig 2). There, the peak for Hb A_2 was revealed at the end of 9 min. retention time with a "shoulder" and continued to the main peak for Hb E. Although the two peaks were not totally separated, concentration for Hb E could be approximated with more confidence from this chromatogram. When the peak area concentration was too low and the shoulder effect was not relevant, it was taken as Hb A_2, ignoring the Hb E content. When the shoulder was prominent and the band in IEF is thick and clear, Hb E concentration was approximated after deducting the area of shoulder from the whole peak.

Hb E and Hb A_2 bands were also not separated in IEF (Fig 3). When the band was thick and clear, IEF provided a strong evidence for presence of Hb E and other abnormal Hb. Hence Hb A_2 band appeared faintly in IEF compared to Hb E band.

According to combined features of DEAE-HPLC and IEF, 78 out of 132 samples (59.1%) had Hb E with the concentration ranging from 10.9% to 88.9%.

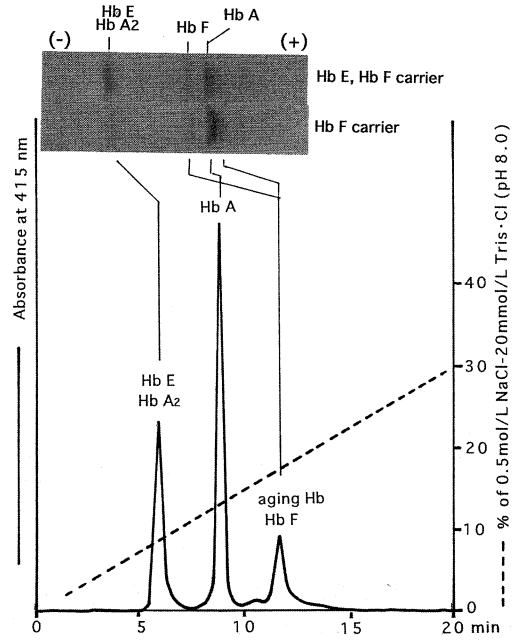

Fig 1. Comparison of Hb bands revealed on IEF plate (pH range: 6-9) of hemolysate prepared from carriers with Hb E and Hb F to Hb elution pattern by DEAE-HPLC of hemolysate prepared from patients with Hb E-β Thal in a usual procedure.

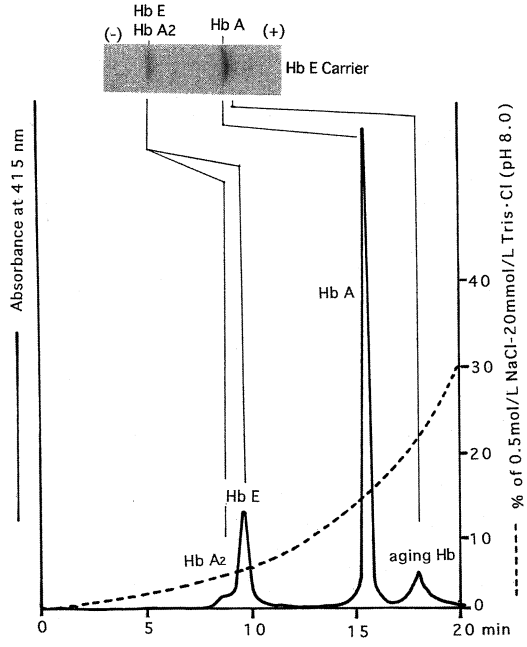

Fig 2. Comparison of Hb bands revealed on IEF plate (pH range: 6-9) of hemolysate prepared from Hb E carrier to Hb elution pattern by DEAE-HPLC of hemolysate in a modified procedure.

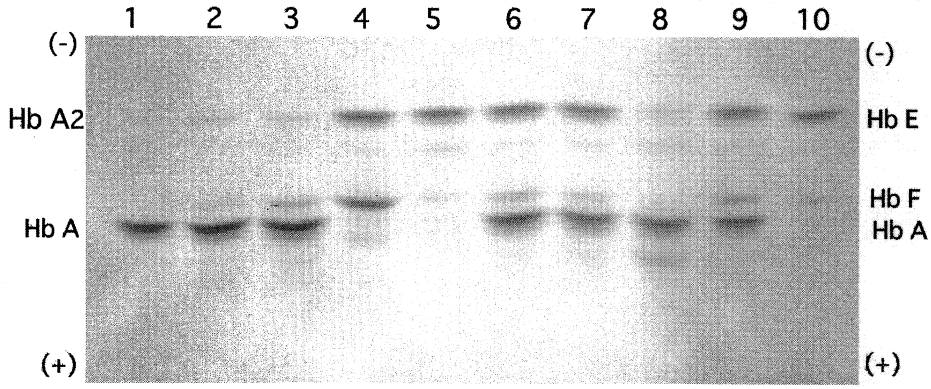

Fig 3. IEF (pH range: 6-9) of the hemolysates prepared from Myanmar patients with Thal.
Lane 1: normal control; Lanes 2 and 3: carriers with high Hb F; Lanes 4 and 10: carriers with Hb E and Hb F, (no Hb A); Lanes 6, 7, and 9: carriers with Hb E, Hb F, and Hb A; Lane 5: homozygote of Hb E; Lane 8: normal Hb pattern with high aging band;

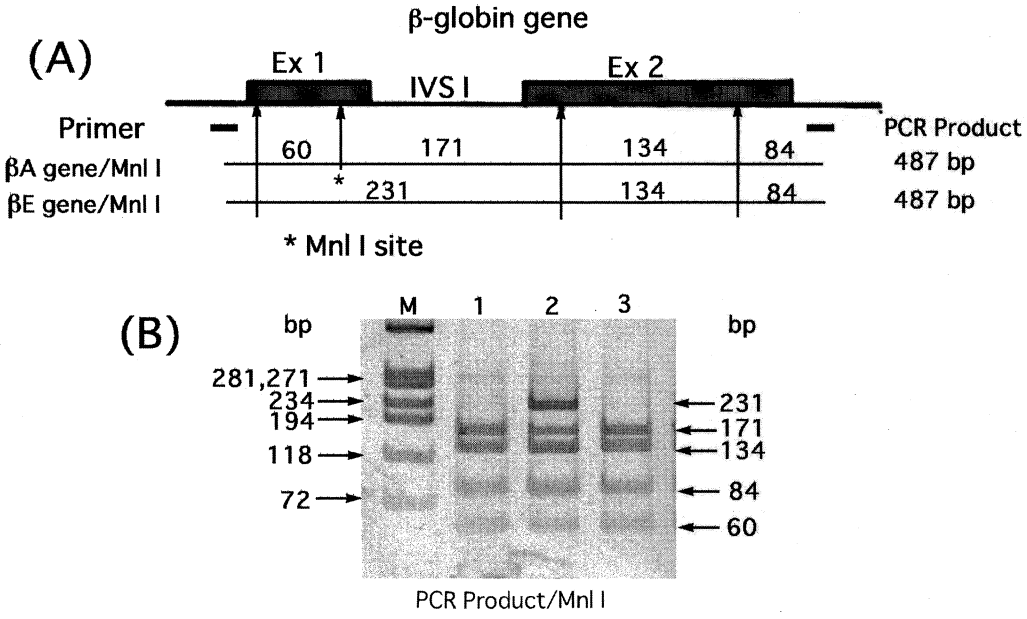

Fig 4. DNA fragment sizes expected by digestion of PCR products with MnI I enzyme (A), and polyacrylamide gel electrophoresis-silver staining analysis of them (B). M: molecular marker (ϕ174/Hae III digestion). Lanes 1 and 3: normal genotype β^A/β^A. Lane 2: heterozygous for β^A/β^E.

There were five samples with the concentration ranging from 5.8%-9.5%, and a decision of presence of Hb E and Hb A_2 either singly or in combination was difficult.

By taking the restriction enzyme analysis finding (Fig 4) as confirmatory, Hb E was observed in 84 patients (63.4%). This finding demonstrates a considerably high prevalence of Hb E among transfusion dependent Thal cases,

in Myanmar. In the Union of Myanmar, there are 1-4.9 births per 1,000 infants with a major hemoglobinopathy according to Modell in 1986.[9] Past studies in Myanmar have confined to the socio-economy,[10] epidemiology and incidence of hemoglobinopathies including Hb E and α Thal traits in community[11-14] and only the clinical and hematological pictures of Hb H disease, β Thal major and Hb E-β Thal disease have been reported.[11,12] Although Hb has been described as a common mutation in Myanmar Thal,[15] it does not represent solely the transfusion dependent group. In our study, sample population is confined to the severe, transfusion dependent group and hospital based. From the epidemiological point of view, this prevalence might reflect to that of the Thal major patients of the whole country because YCH is the largest referral hospital from various parts of the country, Myanmar.

Here we have highlighted the fact that Hb E is the most common mutation interacting with other thalassemic allele(s) giving rise to severe form of Thal in Myanmar. The awareness of Hb E as a common mutant is highly important in prenatal diagnosis for Thal which probably become available in Myanmar, and in the subsequent development of an accurate and efficient Thal control program in the country.

ACKNOWLEDGMENT

This collaborative work is partly a Japan-Myanmar collaborating project, a Grant-in-aid (12672259) by Ministry of Education, Science, Culture and Sports, and partly a Research Project Grant (No. 12-105) from Kawasaki Medical School. We are indebted to Professors Thein Thein Myint and Rai Mra, allowing to collect the samples from the patients. Thanks are due to technicians of Pathology Research Division, DMR, Myanmar, for sample collection and preparation.

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