An Insight into Heterozygous β-Thalassemia, Myanmar

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Accepted for publication on February 3, 2001

ABSTRACT. There are many differential diagnoses of hypochromic microcytic anemia. In Myanmar congenital and acquired hemolytic anemias and iron deficiency anemia are top listed as common causes. The frequency of β-thalassemia is about 4.3% in Myanmar. Since most of heterozygous β-thalassemias (β-thalassemia traits) are asymptomatic and, more importantly, laboratory facility is very limited, the establishment of diagnosis of it is difficult. Here we report 12 cases of Myanmar voluntary subjects with β-thalassemia trait (parents of thalassemia major patients) and the findings of high performance liquid chromatography and isoelectric focusing of their hemolysates. From this small scaled study, we observe that β-thalassemia traits of high Hb A_2 type is more common than that of high Hb F type in Myanmar. Features of δβ-thalassemia heterozygote are not observed.

Key words: High Hb A_2 — High Hb F — β-Thalassemia traits — Myanmar

The diagnosis of heterozygous β-thalassemia (β-thal) is made by the demonstration of an increase in the relative proportion of hemoglobin A_2 (Hb A_2), hemoglobin F (Hb F), or both in a patient whose peripheral blood smear is characterized by hypochromia, basophilic stippling, and the presence of target cells.[1] Since Hb A_2 is decreased in iron deficiency, the above criteria will not be met by the patient whose thal is complicated by iron deficiency. In such a patient, the presence of thal may be suspected from a study of family members and the diagnosis can be documented by the demonstration of increased Hb A_2 after iron therapy.[2] When one or more of the sibbling is having β-thal major (the most severe form of thal which need frequent regular transfusion and have typical clinical features like pallor, easy fatigue, hepatosplenomegaly, malar prominence, short stature and slow or defective growth and development, etc:), both parents always have a form of heterozygous thal, either β- or α-thal and/or abnormal Hb.

Myanmar is situated in Southeast Asia region where thal is highly prevalent. Moreover, iron deficiency is top listed as a causal factor for anemia in that country. More importantly, Myanmar also has a high endemicity of malaria in which hematological changes are similar to those of thal trait and iron deficiency, and mild increase in Hb A_2 and/or Hb F can be seen. Therefore the diagnosis of thal trait is difficult in Myanmar, where laboratory

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facility is also rather limited.

Here, we reported the hemoglobin patterns of 12 voluntary Myanmar subjects, who were the parents of transfusion dependent thal major patients at the day-care-unit of Yangon Children Hospital, Yangon, Union of Myanmar. Hb analysis by use of high performance liquid chromatography (HPLC) with DEAE-5PW column (7.5×7.5 mm, Tosoh Ltd. Co., Tokyo, Japan)[3] and isoelectric focusing (IEF, pH range: 6-9) on polyacrylamide gel plate including carrier ampholytes[4] of their hemolysates was done and the findings were listed in the Table 1.

TABLE 1. Hb pattern detected by DEAE-HPLC and IEF in Myanmar thal traits (relative Hb % was estimated from HPL-chromatogram in terms of area of the peak; peak assignment on HPL-chromatogram, especially aging Hb, was decided by IEF)

Sample No.	Case No.*1	Hb A %	Hb A_2 %	Hb F %	Others/Remarks*2
Normal range			2.2-3.5	<1.5	
1	H19m	90.9	4.9	4.2	aging Hb ca. 1
2	H20f	92.7	4.6	<1	aging Hb ca. 2
3	P1m	90.0	3.8	<1	aging Hb ca. 6
4	P2m	92.9	1.5	<1	aging Hb ca. 5
5	P3m	93.4	3.5	<1	aging Hb ca. 2
6	P4m	67.0	28.4*3	<1	aging Hb ca. 4
7	P5m	90.5	4.5	<1	aging Hb ca. 4
8	T1m	90.9	2.7	1.5	aging Hb ca. 5
9	T2m	91.2	1.3	<1	aging Hb ca. 6
10	T3f	92.7	2.4	<1	aging Hb ca. 4
11	T4m	71.0	25.0*3	<1	aging Hb ca. 3
12	T5m	92.9	3.9	3.2	no aging Hb

*1m or f means mother or father of a child with major thalassemia.
*2Aging Hb content due to the prolonged storage of the sample is estimated from the proportion of peak area on the chromatogram.
*3Including Hb E, which is not separated from Hb A_2 by DEAE-HPLC and IEF. Hb A_2 levels of P4 and T4, estimated by the procedure in the text, were 5.6% and 5.2%, respectively.

The HPL-chromatogram and IEF pattern were observed together for Hb analysis in each case. Hb A, Hb F, Hb A_2 and abnormal Hb (Hb E) were detected in various combination in different concentration. Hb A was detected in all subjects with the concentration of more than 90% of total Hb, except in two subjects (P4 and T4). P4 and T4 had Hb A concentration of 67% and 71% of the total Hb, respectively. Slightly increased value of Hb F (normal range: <1.5%) was found in three subjects but in the remaining 9 subjects it was within the normal range. Hb A_2 concentration (normal range: 2.2-3.5%) was estimated only in 10 subjects and was found to be ranging from 1.3 to 4.9%. Among them, two subjects (P2 and T2) had lower concentration, raising the possibility of associated α-thal ; five subjects (H19, H29, P1, P5, T5) had increased concentration, a feature of β-thal trait.

Hb A_2 could not be quantitated from chromatogram in the two subjects (P4 and T4) since abnormal Hb (Hb E) was associated. A new eluent gradient

system, different from usual linear gradient, was used and it revealed a confirmatory evidence of presence or absence of Hb A_2 (Fig 1). Globin extracted from the hemolysate of these subjects was separated into different chain components by CM-cellulose column chromatography (Fig 2). From these, Hb A_2 (δ-chain) and Hb E (β^E-chain) concentrations were calculated separately. In P4, Hb A_2 was 5.7% and Hb E was 22.7% and in T4, 5.2% and 19.8% for Hb A_2 and Hb E, respectively.

The abnormal Hb found with the Hb A_2 peak was confirmed as Hb E by peptide analysis and amino acid sequence analysis. Its structure detected was β26(B8)Glu → Lys, Hb E.

The Hb pattern of heterozygous β-thal generally was divided into two distinct types; high A_2 β-thal type and high F β-thal or $\delta\beta$-thal type. As the name implies, high Hb A_2 and high Hb F contents were seen in the two β-thal types respectively.[5] In high A_2 β-thal type, Hb A_2 content was 3.5-8.0% and the Hb F content was normal or only slightly elevated (1.5-5.0%). In high Hb F β-thal type, Hb F component ranged 3.5-36% but the level of Hb A_2 was normal. The syndrome having such high Hb F content was known as hereditary persistence of fetal hemoglobin (HPFH). By this definition, there were five subjects (case no. H19, H20, P1, P3, and T5) of high A_2 β-thal type and one Hb F β-thal type (H19). But in case H19, both Hb A_2 and Hb F contents were high and thus a high Hb F β-thal type is unlikely. Reportedly, high Hb A_2 β-thal type was more common than high Hb F β-thal type.[6] Although the sample size was small, the finding from our study on Myanmar β-thal trait was consistent with other reports[6,7] regarding the incidence of high A_2 β-thal type and high F β-thal type.

In addition to the high Hb A_2 and high Hb F genes, there were other β-thal genes which were still less well characterized, eg. both Hb A_2 and Hb F increased genes (case H19 in this study); both Hb A_2 and Hb F decreased genes (like P2 and T2 in this study); or both normal genes (like case P3, T1, and T3 in this study). Such both normal or reduced genes might be a β-thal gene which did not express itself in heterozygous individuals ("silent gene"). These silent genes were expressed whenever an interaction took place with another mutant or allele. For example, case No. P3 might have silent genes because she had Hb A 93.4%, Hb F <1%, and Hb A_2 3.5%. Actually, she had a child of totally transfusion dependent thal major with a hemoglobin phenotype A, F, E (concentration 46.8%, 4.8% and 48.4%, respectively) and a predicted genotype of β^+/β^E or β^0/β^E compound heterozygote (β^+ means that the β-thal gene has a partial defect of β-globin chain synthesis and β^0 means complete defect). Expression of that silent genes was taken place when it was interacted with other forms of mutant or β-thal allele, Hb E.

On the other hand, P2 and T2 subjects who had decreased Hb A_2 levels, were thought to be the carriers of α-thal trait. The genotypes of their children having severe thal major were deduced to be tangled with various complicated α-thal, β-thal, and $\delta\beta$-thal genes.

Another high Hb F β-thal type, designated as heterozygous $\delta\beta$-thal,[8] was not likely in our case (H19 and T5). Hence, the concentration of Hb F was raised only slightly (4.2% and 3.2%, respectively) but marked increas of Hb A_2 (4.9% and 3.9%, respectively). In heterozygous $\delta\beta$-thal, important characteristic features were an increase in Hb F to 5-20%, which was distributed

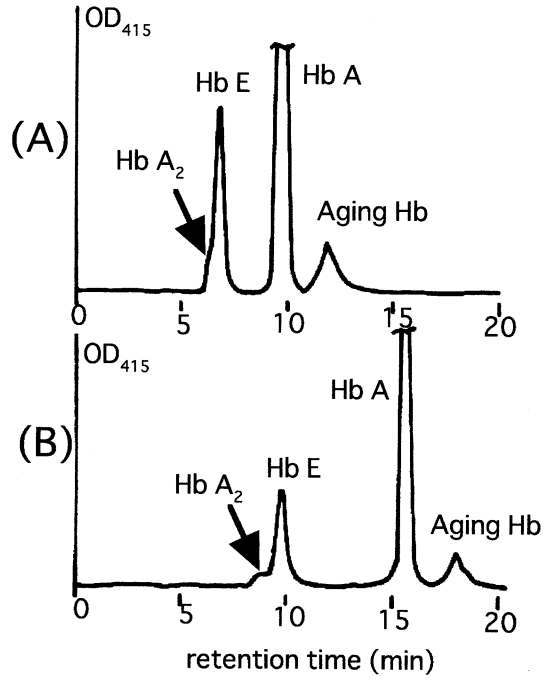

Fig 1. Separation of hemoglobins by DEAE-HPLC, by which the presence of Hb A_2 and Hb E in the peak is revealed at the short retention time (A) and it was confirmed by improved method (B). Quantitation was estimated from each area of Hb peaks. (A) Hb separation by an ordinary method and (B) by an improved method.

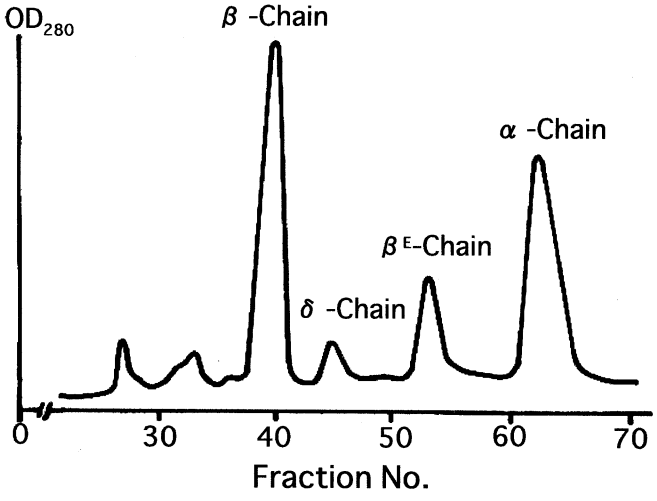

Fig 2. Chain separation of globin prepared from Hb E carrier's hemolysate on CM-celluose column chromatography. The proportion of the areas of δ- and β^E-chains is thought to be equal to that of Hb A_2 and Hb E.

heterogeneously in erythrocytes and the concentration of Hb A_2 was normal or decreased.

Future in-depth studies in Myanmar thals, particularly heterozygous cases who are the carriers of thal genes, are needed for better understanding of clinical and molecular heterogeneity existing there.

ACKNOWLEDGMENT

This work is a part of running Myanmar-Japan Collaboration project funded by Ministry of Education, Science, Culture, and Sports, Japan, and partly supported by a Grant-in-Aid (12672259) from Ministry of Education, Science, Culture, and Sports, Japan, and by a Research Project Grant (12-105) from Kawasaki Medical School. The authors thank to Hauk Kying, Tin Nwe Htwe, and Wah Wah Win, the technicians of Pathology Research Division, Department of Medical Research, Yangon, Union of Myanmar, for sample collection. Additionally, one of the autuors (Ne Win) expresses his gratitude to Dr. S Okada and Dr. K Shimono for their kind arrangements for this study in Kawasaki Medical School.

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