A Study on Molecular Basis of Hypochromic Microcytic Anemia in Myanmar

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ABSTRACT. We studied on molecular characterization of patients with hypochromic microcytic anemia (HMA) from Myanmar. HMA were clarified by Complete Blood Count (CBC) data using Coulter's automated blood cell counter. Hemoglobin analysis was performed by IEF and HPLC to detect abnormal hemoglobins and to estimate the proportions. Multiplex polymerase chain reaction was used for detection of α-thalassemia mutations and, PCR-direct DNA sequencing was also conducted to determine point mutations of globin genes especially focused on $\alpha 2$ globin gene and β globin gene. Among 72 patients, 64 (88.9%) had abnormalities of globin gene and these were α-thalassemia mutations, Hb E and β-thalassemia mutations. The α-thalassemias were detected in 73.3% and their phenotypes were distributed as 36.1%, 30%, and 6.9% for α-thalassemia-2, α-thalassemia-1 and Hb H disease, respectively. The genotypes of these expressed $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{3.7}/-\alpha^{3.7}$, --SEA/$\alpha\alpha$, --SEA/$-\alpha^{3.7}$, --SEA/$\alpha^{CS}\alpha$ and, therefore $-\alpha^{3.7}$ α-thalassemia mutation is the commonest type in Myanmar. Hb E was found in 34.7% of HMA patients and β-thalassemia was detected in only 4 patients in combination with Hb E and α-thalassemia. Prevalence of α-thalassemia mutation is unexpectedly high in this study.

Key words : Hypochromic microcytic anemia — α-thalassemia — PCR — Myanmar

Anemia is a common problem in the world, and is a symptom of disease that requires investigations to determine the underlying etiology. Often, practicing physicians overlook mild anemia. This is similar to failing to seek the etiology of a fever. The purpose of this article is to provide one method of determining an etiology of an anemia. Anemia is strictly defined as a decrease in hemoglobin (Hb) concentration according to the age and sex of the patients. Methods for measuring RBC mass are time consuming, expensive, and usually require transfusion of radio-labeled erythrocytes. Thus, in practice, anemia is usually discovered and quantified by measurement of the RBC count, Hb concentration, and hematocrit (Hct). There are many causes leading to anemia and many types of anemias. During routine investigation of anemias in Myanmar, there is a significant incidence of hypochromic microcytic anemia (HMA), which in the past were mostly assumed to be caused by iron deficiency.[1-3]

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Now we have studied the anemic patients focused mainly on hereditary disorders. Thalassemia is a heterogeneous group of hereditary anemias characterized by reduced or absent production of globin chains. It is very common in Southeast Asia (SEA), the region where another structural β-globin gene variant, hemoglobin E (Hb E), is also prevalent. This abnormal hemoglobin results from a single amino acid substitution of glutamic acid to lysine due to a point mutation (GAG→AAG) in codon 26 of the β-globin gene. The β-thalassemias are seen in Mediterranean, Middle Eastern, and SEA populations. β-Thalassemias are clinically silent in the first months of life and become apparent only after 6-9 months because of cessation of γ-chain production. α-Thalassemias are commonly seen in African and Asian populations including Myanmar.[3,4]

The α-thalassemia syndromes represent a common genetic disorder due to an absence or reduced synthesis of α-globin chain of the hemoglobin. The human α-globin gene cluster is located on chromosome 16p13.3-ter. This cluster contains three functional genes (ζ, $\alpha 2$, $\alpha 1$), three pseudogenes ($\psi\zeta$, $\psi\alpha 2$, $\psi\alpha 1$), and one gene of undetermined function (θ) arranged in the order of "telomere-ζ-$\psi\zeta$-$\psi\alpha 2$-$\psi\alpha 1$-$\alpha 2$-$\alpha 1$-θ-centriosome" within a 40 kb segment. α-Thalassemia frequently results from deletions involving one or both α-globin genes; it is less frequently a result from non-deletional mutations involving one or a few nucleotides within the structural gene.[5]

MATERIALS AND METHODS

Total 72 peripheral blood samples from patients with hypochromic microcytic anemia were collected in North Okkalapa General Hospital, Institute of Medicine (II), from July, 2003 to September, 2004. Hematological data were obtained by automated blood cell counter (Coulter counter). Hemolysates were prepared by ordinary method and DNA extraction was done on these samples by DNA extraction kit (QIAamp kit, Qiagen, Valencia, CA, USA) according to the instruction manual.

Determination of the hemoglobin types was done on hemolysates by isoelectric focussing (IEF) and high performance liquid chromatography (HPLC) (HLC-723 G7, Tosoh Corp. Tokyo, Japan). DNA samples were analyzed by single tube multiplex-PCR (or Gap-PCR) method to detect common α-thalassemia deletions.[2] Mutations of non-deletion types of α-globin gene, and β-blobin gene were detected by PCR-direct DNA sequencing method using the autosequencer (Model 3100, PE-Applied Biosystems Inc.). The synthesized oligonucleotides as sequencing primers were used for detection of the $\alpha 2$-globin and β-globin gene mutations.[6]

RESULTS

Among 72 patients with HMA, 45 cases were female and 27 were male. Their ages range from 7 yr to 66 yrs. On analysis of the hemolysates, Hb E was found in 25 cases (34.7%), and no hemoglobin H (Hb H) was seen in IEF. Twenty four patients were found to be Hb A alone in IEF. On analyzing the hemolysates by HPLC, 12 samples were found to be elevated hemoglobin F (Hb F) and 5 have increased Hb A_2. Normal Hb A_2 level

was found in 41 patients and reduced Hb A_2 level was detected in 26 samples of hemolysates (Table 1).

We found the α-thalassemia mutations in 53 patients (73.6%) and, there were 26 cases of α-thalassemia-2, 22 cases of α-thalassemia-1, and 5 cases of Hb H disease. All cases of α-thalassemia-2 were heterozygotes of $-\alpha^{3.7}$ deletion ($-\alpha^{3.7}/\alpha\alpha$). Among the α-thalassemia-1 cases, 14 patients were homozygotes of $-\alpha^{3.7}$ deletion ($-\alpha^{3.7}/-\alpha^{3.7}$), 4 patients had --SEA deletion (--SEA/$\alpha\alpha$), two cases were homozygotes of Hb Constant Spring (Hb CS) and, another two had to be found heterozygotes of $-\alpha^{3.7}$ and Hb CS ($-\alpha^{3.7}/\alpha^{CS}\alpha$). Four out of 5 Hb H disease had to be --SEA deletion and $-\alpha^{3.7}$ type (--SEA/$-\alpha^{3.7}$) and, heterozygote of --SEA and Hb CS was detected only in one patient with Hb H disease (Table 2). Gene frequencies of α-thalassemia mutation gene were found to be 41.7% for $-\alpha^{3.7}$, 6.3% for --SEA, and 4.9% for α^{CS} (Table 3).

TABLE 1. Genotypes of thalassemias and other globin gene abnormalities detected by multiplex-PCR and DNA sequencing Methods (n=72)

Geneotypes	No(n)	Percentage	Normal Hb A_2	Reduced Hb A_2	Increased Hb A_2
α-thal	39	54.2	19	20	–
Hb E	11	15.3	11	–	–
α-thal-2+Hb E	7	9.7	9	–	1
α-thal-1+Hb E	3	4.2			
α-thal-2+Hb E+β-thal	2	2.8	–	–	2
α-thal-1+Hb E+β-thal	2	2.8			2
Normal	8	11.1	2	6	–
Total	72	100%	41	26	5

TABLE 2. Classificaion of α-thalassemia phenotypes by the genotypes

Phenotypes	Genotypes	No(n)	Frequency
α-thal-2	$-\alpha^{3.7}/\alpha\alpha$	26	21
α-thal-1	$-\alpha^{3.7}/-\alpha^{3.7}$	14	11.3
	--SEA/$\alpha\alpha$	4	3.2
	$\alpha^{CS}\alpha/\alpha^{CS}\alpha$	2	1.6
	$-\alpha^{3.7}/\alpha^{CS}\alpha$	2	1.6
Hb H disease	--SEA/$-\alpha^{3.7}$	4	3.2
	--SEA/$\alpha^{CS}\alpha$	1	0.8

TABLE 3. Proportions of carriers of the three common α-thal mutation alleles (haplotypes) in Myanmar

Haplotypes	Alleles(n)	Proportion
$-\alpha^{3.7}$	60	41.7
--SEA	9	6.3
$\alpha^{CS}\alpha$	7	4.9
Normal $\alpha\alpha$	68	47.2
Total	*124	100%

Hb E was detected in 25 patients with hypochromic microcytic anemia (34.7%) and α-thalassemia mutations were also found in 10 cases with Hb E (13.9%), Four out of 25 patients of Hb E had not only α-thalassemia mutations but also β-thalassemia mutations (Table 1).

DISCUSSION

Among various causes of HMA, thalassemias and hemoglobinopathies are very common in Myanmar and other Southeast Asian countries not only patients but also healthy persons may have at least one type of globin gene mutations. In patients with HMA, the total frequency of the carriers of thalassemias and abnormal hemoglobins was as high as 88.9%. Out of the 72 HMA cases, the α-globin gene mutations resulting in various phenotypes of α-thalassemias (73.6%) was unexpectedly much more common than other hemoglobin abnormalities. There are various phenotype of this; α-thalassemia-2 (36.4%), α-thalassemia-1 (30.5%) and Hb H disease (7%). Their genotypes are distributed as follows: (1) $-\alpha^{3.7}/\alpha\alpha$ in α-thalassemia-2, (2) $-\alpha^{3.7}/-\alpha^{3.7}$, --SEA/$\alpha\alpha$, $\alpha^{CS}\alpha/\alpha^{CS}\alpha$, and $-\alpha^{3.7}/\alpha^{CS}\alpha$ in descending order in α-thalassemia-1, and (3) --SEA/$-\alpha^{3.7}$ and --SEA/$\alpha^{CS}\alpha$ in Hb H disease. Among these different types of mutations, $-\alpha^{3.7}$ deletion is the commonest type of α-globin gene mutations and it is similar to previous published data.[7] Among the three subtypes of $-\alpha^{3.7}$ deletions; which are classified to type I, II, and III, by the different positions of the 3.7 kb deletion, we found that the majority is type I in Myanmar.[8,9] The majority of patients with Hb H disease are heterozygotes of --SEA and $-\alpha^{3.7}$ deletions. The secound most common type is --SEA deletion which is also common in SEA region, 36.4% in Southern China.[10] In this area, --SEA deletion is the commonest type of α-thalassemia mutation, that is different when compared with Myanmar.[1,11] Therefore, as in other countries in SEA region, deletion types of α-thalassemia mutation are much more common than non-deletion types such as point mutation.[12] But in cases of β-globin gene abnormalities, point mutations are main types in comparing deletions. Among them, point mutation at codon 26 of β-globin gene resulting Hb E comprises 15.3% of patients in this study, so it is also common even in cases of HMA. According to previous study in Myanmar, Hb E was detected in 60% of population (n=115).[2]

About 19% (10/53) of α-thalassemia also had an associated Hb E and therefore rather high prevalence of Hb E-α thalassemia heterozygotes can be decided in Myanmar. We also found that α^{CS} gene (Hb CS) is not uncommon (5/72) (7%) in this study and its frequency is about 8% in SEA.[5]

This study was conducted as an attempt to detect molecular defects focused on thalassemias and hemoglobinopathies only in patients with HMA. The findings point out that not only patients but also general population should be screen for thalassemias and hemoglobinopathies because these will be one challenging health problem soon in Myanmar. A further problem is that great heterogeneity in expression is found even within the same genotype, so it is very difficult to make generalizations concerning the clinical picture of the disease. Thalassaemia is a good candidate disease for control by preventive genetic programs in developing countries and now

many countries have been taking the action on the control and prevention of common genetic disorders.

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