Changes in HbA_2 Levels May Signify Hemoglobin Defects in Infants

To the editor:

The hemoglobin profile has been utilized as a clue for the diagnosis of thalassemia and hemoglobinopathy. Fetal hemoglobin (HbF, $\alpha_2\gamma_2$) is highly expressed as the major functional hemoglobin during the fetal stage. Following hemoglobin switching, HbF declines to less than 1% of the total hemoglobin within one year of age. Adult hemoglobin (HbA, $\alpha_2\beta_2$) therefore predominates over total hemoglobin in combination with 2-3% of HbA_2 ($\alpha_2\delta_2$) and trace amounts of HbF throughout adult life. Hemoglobin can routinely be analyzed using chromatography or electrophoresis. Relative alterations of these hemoglobin types have been shown to be associated with abnormal globin production in adults. For instance, hemoglobin E (HbE, $\alpha_2\beta_2^{26Glu\rightarrow Lys}$), the hallmark hemoglobinopathy of Southeast Asia, exists in a range from 25–90% of the total hemoglobin in adults with HbE inheritance; however, HbE levels present less than 25% of total hemoglobin in heterozygous HbE patients who co-inherit with α-thalassemia. Moreover, HbA_2 is markedly raised between 3.5 and 9.9% in adult heterozygous β-thalassemia. In contrast, relative changes in hemoglobin types are unnoticeable by routine hemoglobin analysis in adults with one or two α-globin gene defects.[1] To broaden the knowledge and illustrate the effects of globin disorders on hemoglobin profile in infants, leftover blood samples from 142 unrelated individuals aged between 8 and 12 months with a mean corpuscular volume (MCV) less than 80 fL were subjected to thalassemia screening and diagnosis. Hemoglobin analysis was performed regarding the Bio-Rad Variant II Hemoglobin Testing System with β-Thalassemia Short Program (BioRad, Hercules, CA). The diagnosis of thalassemia was performed according to standard diagnostic guidelines used in Thailand.[2-4] In addition, common deletion types of α^+-thalassemia ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) and α^0-thalassemia ($--^{SEA}$ and $--^{THAI}$) were genotyped as described in previous studies.[5-6] The protocols in this study were approved by the ethics committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (ethical approval reference number AMSEC-63EM-001). The results revealed that 59 (41.5%) were negative for the common thalassemias (hereafter so called normal),37 (26.1%) were heterozygous for β-thalassemia, 19 (13.4%) were heterozygous for HbE, 18 (12.7%) were heterozygous for α-thalassemia 1, and 9 (6.3%) were deletional HbH disease caused by compound heterozygous α-thalassemia 1 with α-thalassemia 2 (Table 1). Complete blood count (CBC) showed hypochromic microcytic anemia with reduced total Hb level, Hct, MCV, and mean corpuscular hemoglobin (MCH) (Table 1). On average, the hemoglobin profile of normal infants was similar to that of normal adults, suggesting that the hemoglobin switching had completed in these subjects. Conversely, a significant increase in HbA_2 and HbF levels was observed in infants with heterozygous β-thalassemia and heterozygous HbE (Table 1). The elevated HbA_2 levels were similar to those of adults with heterozygous β-thalassemia. Regardless of age, this suggested that increased HbA_2 levels determine the inheritance of β-thalassemia. Interestingly, elevated HbF levels were absent in adults with either heterozygous β-thalassemia or heterozygous HbE,[7-8] suggesting a developmental stage-specific effect. This finding is comparable to previous studies in which the delayed HbF to HbA switching is remarkably shown in infants with β-globin defects.[9,10] Although the mechanisms underlining HbF to HbA switching are unclear, the prolonged HbF levels in infants with a β-globin defect maybe due to the primary compensation of β-like globin gene expression and total hemoglobin during development in affected infants. Similar to those of adults with homozygous β^0-thalassemia and compound heterozygous β^0-thalassemia with HbE disease, the increase in γ-globin gene expression has been shown to associate with milder clinical manifestations as it is able to substitute for the inadequate β-globin gene expression and yields increased HbF levels. In contrast to β-globin gene defects, heterozygous α^0-thalassemia demonstrated comparable hemoglobin types to the normal in our finding. Despite insignificance, infants with two α-globin gene defects displayed reduced HbA_2 and modestly increased HbF levels in the previous study.[9] The decrease in HbA_2 levels was clearly noticed in infants with three α-globin gene defects or deletional HbH disease in our study. Together, the results suggested that HbA_2 levels may be considered as valuable markers for the inheritance of globin gene defects in infants.

TABLE 1. Hematological data of the infants participated in this study.

Characteristic	Age (Month), Mean ± SD	Sex (No.), Boy/Girl	RBC (10^{12}/L), Mean ± SD	Hb (g/dL), Mean ± SD	Hct (%), Mean ± SD	MCV (fL), Mean ± SD	MCH (pg), Mean ± SD	MCHC (g/dL), Mean ± SD	HbA2/E (%), Mean ± SD	HbF (%), Mean ± SD	HbA (%), Mean ± SD	Hb Bart's	HbH
Normal (n = 59)	11.6 ± 1.0	27/32	5.1 ± 0.5	9.9 ± 1.2	32.0 ± 3.5	62.5 ± 4.4	19.3 ± 2.1	30.9 ± 1.6	2.6 ± 0.4	1.1 ± 0.8	91.5 ± 5.8	Absent	Absent
Heterozygous β-thalassemia (n = 37)	12.0 ± 0.2	21/16	5.6 ± 0.5	10.0 ± 0.7	31.9 ± 2.4	56.8 ± 3.0	17.9 ± 1.1	31.5 ± 1.4	5.5 ± 0.5	6.1 ± 3.8	83.3 ± 6.8	Absent	Absent
Heterozygous HbE (n = 19)	11.9 ± 0.2	11/8	5.0 ± 0.6	10.6 ± 2.2	31.8 ± 4.9	64.1 ± 6.1	21.3 ± 3.4	33.1 ± 3.8	24.7 ± 4.2	4.2 ± 2.4	66.7 ± 7.1	Absent	Absent
Heterozygous α^0-thalassemia ($--^{SEA}/\alpha\alpha$) (n = 18)	12.0 ± 0.0	10/8	6.0 ± 0.5	10.4 ± 1.2	34.0 ± 3.6	57.2 ± 6.2	17.5 ± 2.2	30.5 ± 1.3	2.5 ± 0.3	1.2 ± 0.8	92.7 ± 5.5	Absent	Absent
HbH disease ($--^{SEA}/-\alpha^{3.7}$) (n = 9)	12.0 ± 0.0	4/5	5.8 ± 0.6	9.1 ± 0.7	30.8 ± 2.7	53.0 ± 2.9	15.7 ± 1.5	29.5 ± 1.8	1.6 ± 0.5	1.4 ± 1.1	92.7 ± 3.5	Present	Present

Abbreviations: RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

REFERENCES

1. Akhavan-Niaki H, Youssefi Kamangari R, Banihashemi A, Kholghi Oskooei V, Azizi M, Tamaddoni A, et al. Hematologic features of alpha thalassemia carriers. Int J Mol Cell Med 2012;1:162-7.
2. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. Bull World Health Organ 2004;82:364-72.
3. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratanasiri T, Sanchaisuriya P, Changtrakul Y, et al. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy: an alternative approach to electronic blood cell counting. Am J Clin Pathol 2005;123:113-8.
4. Karnpean R, Fucharoen G, Pansuwan A, Changtrakul D, Fucharoen S. A proficiency testing program of hemoglobin analysis in prevention and control of severe hemoglobinopathies in Thailand. Clin Chem Lab Med 2013;51:1265-71.
5. Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of alpha-thalassaemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions. Br J Haematol 2000;108:295-9.
6. Pornprasert S, Wiengkum T, Srithep S, Chainoi I, Singboottra P, Wongwiwatthananukit S. Detection of alpha-thalassemia-1 Southeast Asian and Thai type deletions and beta-thalassemia 3.5-kb deletion by single-tube multiplex real-time PCR with SYBR Green1 and high-resolution melting analysis. Korean J Lab Med 2011;31:138-42.
7. Yamsri S, Sanchaisuriya K, Fucharoen G, Sae-Ung N, Fucharoen S. Genotype and phenotype characterizations in a large cohort of beta-thalassemia heterozygote with different forms of alpha-thalassemia in northeast Thailand. Blood Cells Mol Dis 2011;47:120-4.
8. Sae-ung N, Srivorakun H, Fucharoen G, Yamsri S, Sanchaisuriya K, Fucharoen S. Phenotypic expression of hemoglobins A(2), E and F in various hemoglobin E related disorders. Blood Cells Mol Dis 2012;48:11-16.
9. Kingchaiyaphum B, Sanchaisuriya K, Fucharoen G, Chaibunruang A, Hess SY, Hinnouho GM, et al. Hemoglobins F, A2 , and E levels in Laotian children aged 6-23 months with Hb E disorders: Effect of age, sex, and thalassemia types. Int J Lab Hematol 2020;42:277-83.
10. Winichagoon P, Svasti S, Winichagoon P, Chitchumroonchokchai C, Fucharoen S. Expression of betaE and gamma-globin genes in infants heterozygous for hemoglobin E and double heterozygous for hemoglobin E and alpha-thalassemia. Haematologica 2007; 92:702-3.

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