Journal of Korean Medical Science
Vol. 7, No. 4, 385-388 December 1992

A Family Case of β-Thalassemia Minor and Hemoglobin Queens: α34 (B15) Leu-Arg

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We report a Korean family case of β-thalassemia minor and Hb Queens. This is the first case report of Hb Queens in Korea. A 43-year-old male and his four family members had β-thalassemia minor which is very rare in Korea. Incidentally, an α chain variant with a high isoelectric point was also found in two other family members without clinical problems and was finally identified as α34 (B15) Leu-Arg or Hemoglobin Queens.

Key Words: *β-thalassemia minor, Hb Queens*

INTRODUCTION

Thalassemia has the highest incidence in people of Mediterranean, African, Middle and South-East Asian origin. However it is very rare in Korea, and the first Korean case of β-thalassemia minor was reported in 1988 (Koo et al., 1988).

Hemoglobin Queens, α34 (B15) Leu-Arg, was first reported in 1979 (Tatsis, 1979). It has been found in oriental races: Korean, Japanese, Chinese and Vietnamese (Sugihara et al., 1982; Moo-Penn et al., 1982; Yongsuwan et al., 1987).

Recently, the authors experienced a family case of β-thalassemia minor and Hb Queens. Five family members including the patient had anemia and other laboratory findings consistent with β-thalassemia minor. Two other family members without β-thalassemia had an α chain variant, Hb Queens but they had no clinical symptoms.

CASE REPORT

The patient was a 43-year-old Korean male who presented with symptoms of general malaise and dizziness for the last five years, and was found to be icteric with hepatosplenomegaly. Hematological investigations revealed: Hb 8.3 g/dL; mean corpuscular volume (MCV) 76.4 fl; mean corpuscular hemoglobin (MCH) 23.7 pg and reticulocytes 7.1%. Other laboratory findings showed total bilirubin 5.1mg/dL; direct bilirubin 0.7mg/dL; LDH 485 U/L; Hb A_2 4.3% and Hb F 9.9% with cellulose acetate electrophoresis. Serum iron and total iron binding capacity were within normal ranges. Peripheral blood smear showed anisopoikilocytosis, hypochromia, target cells and basophilic stippling. An examination of bone marrow revealed increased erythropoiesis with basophilic stippling and many sea-blue histiocytes.

Eight family members were studied (Fig. 1) and four of them had anemia and similar laboratory findings (Table 1). Incidentally, an α chain variant with a high

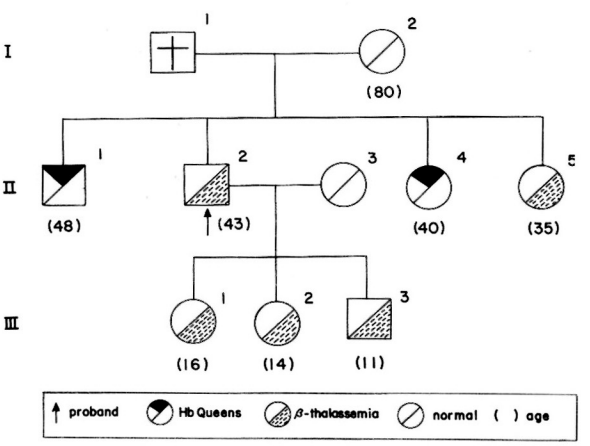

Fig. 1. Pedigree of the family with β-thalassemia and Hb Queens

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isoelectric point was found in two other family members without anemia and studied as follows.

Table 1. Laboratory findings of the family members with anemia

	II-2	II-5	III-1	III-2	III-3
RBC ($10^6/\mu L$)	3.64	4.33	5.09	4.64	5.95
Hb (g/dL)	8.1	9.5	10.0	9.3	9.4
Hct (%)	29.1	30.4	37.5	34.2	36.4
MCV (fL)	78.0	70.0	74.0	73.0	61.0
MCH (pg)	22.3	21.0	21.4	20.0	17.5
MCHC (g/dL)	28.5	29.9	28.8	27.4	27.5
Reti (%)	7.4	4.1	2.8	3.0	3.0
Bil (mg/dL)	5.2	0.7	1.4	1.8	1.1
LDH (U/L)	485	NT	311	192	218
Hp (mg/dL)	<38	<38	<38	<38	<38
Hb A_2 (%)	4.3	4.9	4.9	5.5	5.8
Hb F (%)	9.9	3.9	6.0	8.0	2.1
(HPLC)	13.1	NT	7.5	9.6	2.8
Heinz body	+	NT	±	±	±
PBS	anisopoikilocytosis, hypochromia, target cells basophilic stippling				

NT: not tested Reti: reticulocyte count
Bil: bilirubin Hp: haptoglobin
HPLC: high performance liquid chromatograph
PBS: peripheral blood smear

METHODS AND RESULTS

Abnormal hemoglobin was detected on cellulose acetate electrophoresis (pH 8.6, Tris-borate-EDTA buffer) and isoelectrofocusing (approximately 0.2 pH units/cm) (Basset et al., 1978). The α chain was separated by urea CM-cellulose column chromatography (Clegg et al., 1968). The abnormal α chain was digested with trypsin (enzyme/substrate ratio w/w 1:100, pH 8-9, 4 hours at 37°C) and analysed by cation exchange HPLC on a SP-2SW column (Tosoh) (Ohba et al., 1989). The abnormal tryptic peptide was rechromatographyed on an ODS column (μBondapak C_{18} column).

The patient's brother (II-1) and sister (II-4) appeared healthy and had normal results in routine laboratory studies. But Hb electrophoresis on cellulose acetate and isoelectrofocusing revealed an abnormal band (Fig. 2 A, B). In urea CM-cellulose column chromatography the abnormal α chain eluted later than the normal α chain, which was about 15% of the total α chain (Fig. 3). In the analysis of abnormal hemoglobin using cation exchange HPLC, αT-5 was missing and there was an abnormal peak eluted faster; also, the peak for αT-14 seemed abnormally 'fat' (Fig. 4). The fat αT-14 peak actually contained another abnormal

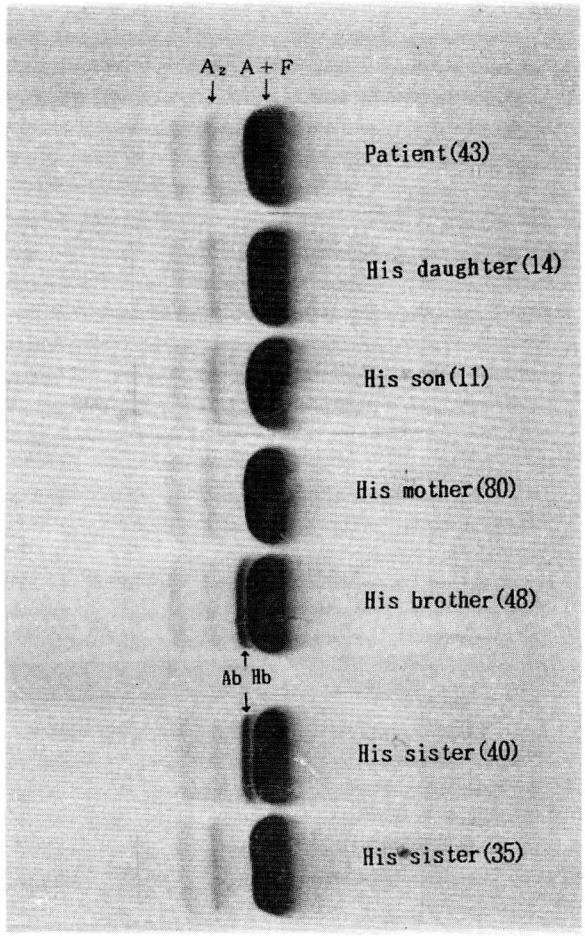

Fig. 2. A. Hemoglobin electrophoresis on cellulose acetate shows abnormal hemoglobin bands in the patient's brother and sister.

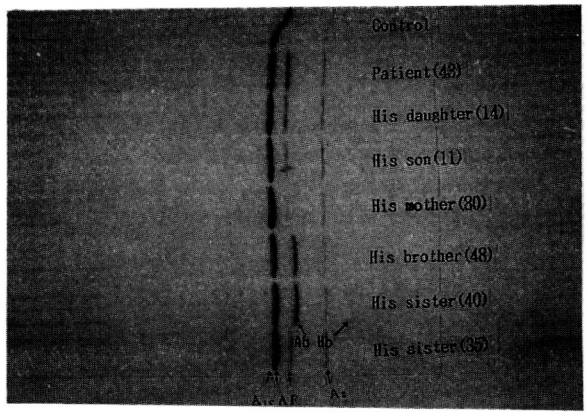

Fig. 2. B. Isoelectrofocusing shows abnormal hemoglobin bands in the patient's brother and sister.

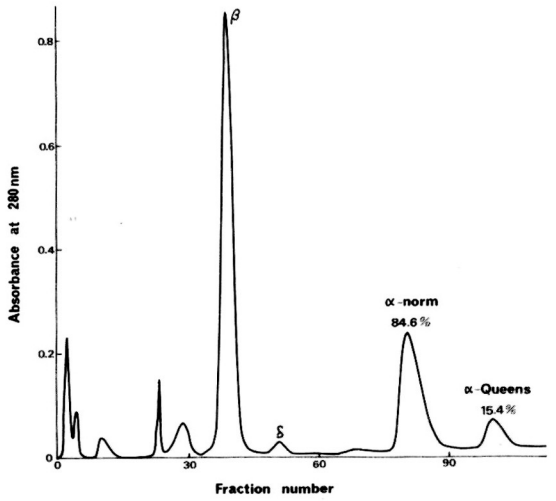

Fig. 3. α-Chains prepared by urea CM-cellulose column chromatography. The abnormal α-chain eluted later than the normal α-chain. It comprised about 15% of the total α-chain.

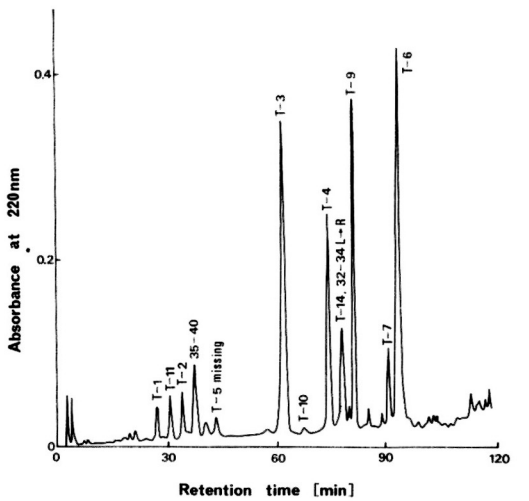

Fig. 4. The abnormal α-chain was digested with trypsin and studied by cation exchange HPLC on a SP-2SW column. αT-5 was missing and there was an abnormal peak eluting faster; also, the peak for αT-14 seemed abnormally 'fat'.

	32	35	40
Normal	Met-Phe-Leu-Ser-Phe-Pro-Thr-Thr-Lys		
Abnormal αT-5		-Arg-	

Fig. 5. Amino acid sequence of normal hemoglobin and Hb Queens

peptide which was eluted later than αT-14 (but faster than the other abnormal peptide) in rechromatography. The composition of the first abnormal tryptic peptide was Thr 2.0, Ser 1.0, Pro 0.8, Phe 1.2 and Lys 1.1 (molar ratio) and that of the second was Met 0.2 (oxidative loss during hydrolysis), Phe 1.1 and Arg 0.9. Summing up, the composition of the two peptides agreed with αT-5 except for the absence of leucine and the presence of arginine. Thus, substitution of Arg for Leu at position 34 of the α chain was deduced (Fig. 5).

DISCUSSION

It has been known that thalassemia has the highest incidence in people of Mediterranean, African, Middle and South-East Asian origin. Korea was not included in the thalassemia belt (geographic distribution of thalassemia) (Chernoff, 1959) but, the first case of β-thalassemia minor was reported in 1988 (Koo et al., 1988). Thus any relation to other areas of high incidence could be traced. In this case, the patient and his family members with anemia were consistent with β-thalassemia minor. Favoring this diagnosis were mild anemia, reduced Hb levels without decreased red cell counts, low MCV and MCH, typical red cell morphology, and elevated Hb A_2 levels (ranged from 4.3% to 5.8%). In some of the family members (the patient and his two daughters) levels of Hb F were unusually high, while in other (his sister and son) they were not. Elevated serum LDH and low levels of haptoglobin, especially in the patient, might indicate the presence of rather severe hemolysis which was unusual for a β-thalassemia trait. Probably the high Hb F level reflected the degree of hemolysis and selective survival of F cells. However, Hb F level is variably increased in β-thalassemia traits and the frequency or degree of its elevation is different depending on the reporters.

Hemoglobin Queens, the substitution α34 (B15) Leu-Arg, was accidentally found in the family study. This hemoglobinopathy, which was first found in a 16-year old Korean girl, was reported by Tatsis in Queens Hospital Center, Queens, New York (Tatsis, 1979). Since then it has been found in oriental races-Japanese, Chinese and Vietnamese (Sugihara et al., 1982; Moo-Penn et al., 1982; Yongsuwan et al., 1987). So we can expect the new cases of Hb Queens in future, though this case report is first in Korea. Hb Queens seems to have no clinical relevance, as in this case, because the substitution of arginine for leucine would not be expected to create any major disruption to the structure and does not exhibit abnormal functional properties (Moo-Penn et al., 1982). Low concentrations of Hb

Queens in heterozygous carriers may be explained by the mutation in the $\alpha 1$ locus whose expression is lower than that in the $\alpha 2$ locus (Liebhaber at al., 1986), and/or by its slight instability (Yongsuwan et al., 1987).

It is known that thalassemias and hemoglobinopathies are very rare in Korea, but we report a family case of β-thalassemia minor and Hb Queens. We expect careful diagnosis and intensive studies might reveal many new cases found in Korea.

REFERENCES

Basset P, Beuzard Y, Garel MC & Rosa J: *Isoelectric focusing of human hemoglobin: Its application to screening, to the characterization of 70 variants, and to the study of modified fractions of normal hemoglobin. Blood 51:971-982, 1978.*

Chernoff AI: *The distribution of the thalassemia gene: a historical review. Blood 14:899-912, 1959.*

Clegg JB, Naughton MA & Weatherall DJ: *Separation of the α and β-chains of human hemoglobin. Nature 219:69-70, 1968.*

Koo MS, Lee HS, Ahn YM, Cho HI, Kim JQ & Kim SI: *A case of β-thalassemia minor in a Korean family. Korean J Clin Pathol 8:153-160, 1988.*

Liebhaber SA, Cash FE & Ballas SK: *Human α-globin gene expression. The dominant role of the $\alpha 2$-locus in mRNA and protein synthesis. J Biol Chem 261:15327-15333, 1986.*

Moo-Penn WF, Jue DL, Johnson MH, McGuffey JE, Simpkins H & Katz J: *Hemoglobin Queens: $\alpha 34$ (B15) leu-arg, Structural and functional properties and its association with Hb E. Am J Hematol 13:323-327, 1982.*

Ohba Y, Imai K, Kumada I, Ohsawa A & Miyaji T: *Hb Moriguchi or $\alpha 2\beta 297$ (FG4) His->Tyr substitution at the $\alpha 1$-$\beta 2$ interface. Hemoglobin 13:367-376, 1989.*

Sugihara J. Imamura T, Yamada H, Imoto T, Matsuo T, Sumida I & Yanese T: *A new electropholetic variant of Hemoglobin (Ogi) in which a leucine residue is replaced by an arginine residue at position 34 of the α-chain. Biochim Biophys Acta 701:45-48, 1982.*

Tatsis B: *Hemoglobin Queens ($\alpha 34$ (B15) Leu-Arg): A New variant at the $\alpha 1\beta 1$ contact. Blood, Suppl1 54:61a, 1979.*

Yongsuwan S, Svasti J & Fucharoen S: *Decreased heat stability found in purified hemoglobin Queens (alpha34 (B15) Leu-Arg) Hemoglobin 11:567-570, 1987.*