Case Report

Hemoglobin J-Singapore [α79(EF8)Ala→Gly, GCG>GGG] in a Pregnant Thai Woman

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Hemoglobin [Hb] J-Singapore [α79(EF8)Ala→Gly, GCG>GGG] is a very rare α-globin chain variant. In the present study, the authors reported, for the first time, the Hb J-Singapore in a 31-year-old Thai pregnant woman. She was seen by an obstetrician at her fifteenth week of gestation. Based on routine antenatal thalassemia and hemoglobinopathy screening, her Hb analysis was performed by high performance liquid chromatography [HPLC] and the abnormal Hb peak with a value of 23.6% was observed at the retention time of 1.83 minutes. The abnormal Hb peak was also found at zone 12 of capillary electrophoresis [CE] electrophoregram, which was similar to the peak of Hb Bart's and Hb J-Buda. The direct DNA sequencing revealed the GCG>GGG mutation at codon 79 of α2-globin gene as previously described for Hb J-Singapore. In addition, the developed multiplex allele specific polymerase chain reaction [MAS-PCR] showed the 632 bp amplified fragment from Hb J-Singapore allele. Thus, the knowledge and understanding of this hemoglobinopathy will be used to assist in diagnosis, management, and counseling for needed patients.

Keywords: J-Singapore, CE, Diagnosis, HPLC, Multiplex allele specific-PCR

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Hemoglobin [Hb] J-Singapore [α78(EF7)Asn→Asp; α79(EF8)Ala→Gly], is a fast moving variant that was reported to contain two amino acid substitutions, asparagine to aspartic acid at position α78, and alanine to glycine at position α79. It was firstly observed in 1972 by Blackwell et al in a Malaysian family living in Singapore[1]. The fast moving α-chain Hb variant named Hb J-Singa [α78(EF7)Asn→Asp], which had the same amino acid substitution as one of the two present in Hb J-Singapore, was found in a French-Acadian family living in Eastern Canada[2]. Since 2007, there has been only one report of the Hb J-Singapore. It was found in a 27-year-old female of Malaysian origin living in Ireland, during a routine ante-natal hemoglobinopathy screening. It showed only one mutation of C to G transversion at codon 79 on the α_2-globin gene [α79(EF8)Ala→Gly, GCG>GGG][3]. In the present study, the authors reported, for the first time, the discovery of Hb J-Singapore in a Thai pregnant woman.

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Case Report

A 31-year-old Thai pregnant woman was seen by an obstetrician at Uttaradit Hospital, Uttaradit, Thailand when she was in her fifteenth week of gestation. Based on a routine antenatal thalassemia and hemoglobinopathy screening, her blood sample was collected with ethylenediaminetetraacetic acid [EDTA] as anticoagulant and the hematological parameters were measured by the ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostic, Deerfield, IL, USA). Values observed were white blood cell [WBC] 7.90x10^9 cells/L, red blood cell [RBC] 3.71x10^{12} cells/L, Hb 120 g/L, packed cell volume [PCV] 0.36 L/L, mean corpuscular volume [MCV] 98.3 fL, mean corpuscular hemoglobin [MCH] 32.4 pg, mean corpuscular hemoglobin concentration [MCHC] 330 g/L, red cell distribution width [RDW] 13.6%, and platelet 232x10^9/L. The Hb analysis was performed by using high performance liquid chromatography [HPLC] (VARIANT II, β-thalassemia Short Program, Bio-Rad Laboratories, Hercules, California, USA). The results showed that the levels of HbA, A_2, and F were 68.6%, 2.5%, and 0.5%, respectively. In addition, an abnormal Hb peak with a value of 23.6% of the total Hb was observed at the retention time of 1.83 minutes (Figure 1A). The blood sample was also sent to the Associated Medical

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Sciences [AMS] Clinical Service Center, Chiang Mai University, Chiang Mai, Thailand for Hb analysis by using capillary electrophoresis [CE] (Capillarys™ 2 Flex Piercing, Sebia, Norcross, Georgia, USA). On CE electrophoregram, the abnormal Hb peak with a value of 20.9% of the total Hb was presented at zone 12 with the retention time of 100 to 110 seconds (Figure 1B). Furthermore, the genomic DNA was extracted from the blood sample by using the NucleoSpin® kit (Macherey-Nagel, KG, Duren, Germany) according to manufacturers' instructions. The DNA was stored at -20°C until used. The real-time polymerase chain reaction [PCR] with SYBR Green 1 and high resolution melting [HRM] analysis[4,5] for detection of the α-thalassemia-1 Southeast Asian [SEA] and Thai type deletions was performed, at the same time as the Hb analysis by CE method was carried out. The multiplex Gap-PCR for detection of α-thalassemia-2 ($-\alpha^{3.7}$ and $-\alpha^{4.2}$ kb deletions)[6,7] and the multiplex allele specific [MAS]-PCR for diagnosis of Hb constant spring [CS] and Hb Pakse'[8] were also performed in this case. The negative analysis results for α-thalassemia-1 and -2 deletions and Hb CS and Hb Pakse' mutations were observed. To identify the abnormal Hb, the PCR amplification of the α-globin gene was accomplished and then the direct DNA sequencing of the amplified product was performed on an ABI PRISM™ 3130 XL analyzer (Applied Biosystems, Foster City, CA, USA). The results showed the molecular defect causing abnormal Hb resulting in a C to G transversion at the second position of codon 79 (GCG>GGG) of α_2-globin gene (Figure 2) that leads to a substitution of glycine for alanine as previously described for Hb J-Singapore[1].

Because the peaks of Hb J-Singapore and Hb J-Buda [α61(E10) Lys→Asn, AAG>AAT] are eluted at the same retention time on HPLC chromatogram and the same zone on CE electrophoregram[9], the MAS-PCR for identification of these Hb variants was developed. The Hb J-Singapore specific reverse primer, SP05 (5'-TCG CTC AGG GCG GAC AGC C-3') was used with the forward common primer, C1 (5'-TGG AGG GTG GAG ACG TCC TG-3') to produce a 632 bp while the Hb J-Buda specific forward primer, SP02 (5'-GTT AAG GGC CAC GGC AAG AAT-3') was used with the reverse common primer B (5'-GAG GCC CAA GGG GCA AGA AGC AT-3') to produce a 455 bp meanwhile the forward common primer C1 and reverse common primer B were used to generate the 975 bp to serve as internal control fragment (Figure 3). The MAS-PCR mixture (50 µL) contains 50 to 200 ng genomic DNA, 45 pmoles of primer C1, 60 pmoles of

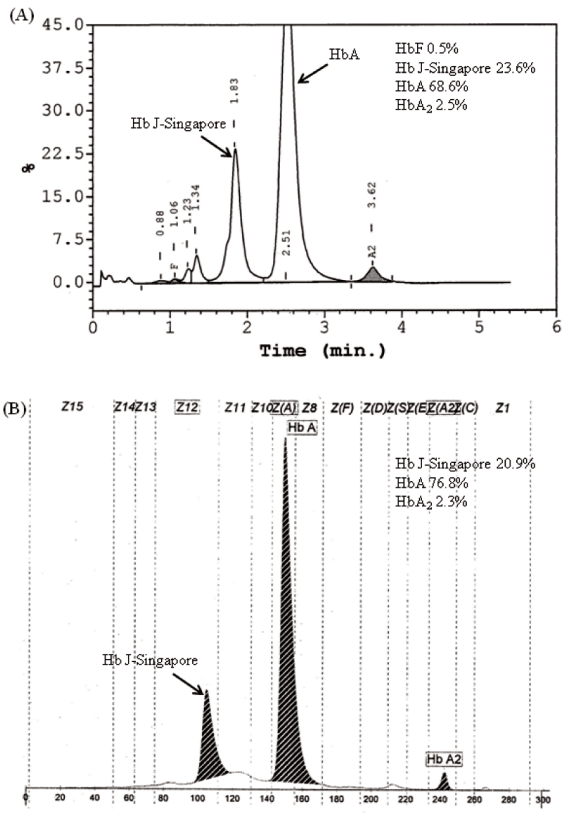

Figure 1. The HPLC chromatogram (A) and CE electrophoregram (B) of the propositus.

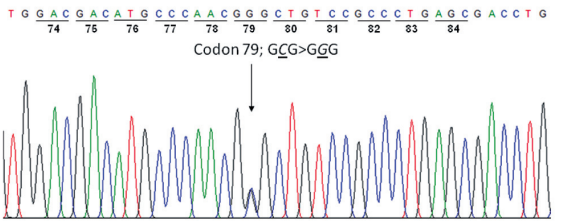

Figure 2. Representative of DNA sequence derived from the propositus. The arrow indicates the heterozygote of Hb J-Singapore nucleotide mutation in the α_2-globin gene.

primer SP02 and 30 pmoles of primers SP05 and B, 200 µM dNTPs and 1.5 unit Taq DNA polymerase (New England Biolabs Inc., Ipswich, MA, USA) in 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin. The amplification reaction was carried out on a Mycycler Thermalcycler (Cyclerus personalis, Bio-Rad, USA). After an initial heating at 94°C for three minutes, the reaction was followed by 10 cycles of 94°C for 30 seconds, 65°C for 30 seconds, 72°C for two minutes, and 20 cycles of 94°C for 30

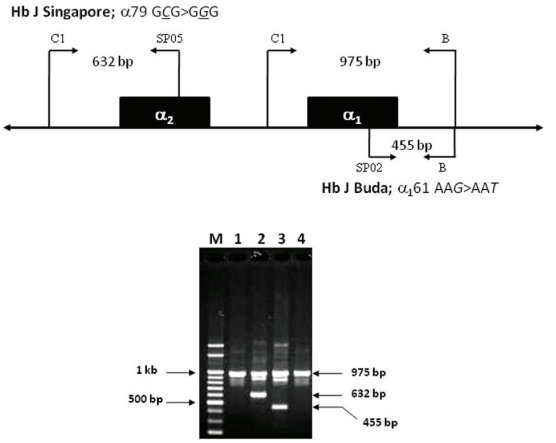

Figure 3. The multiplex allele specific polymerase chain reaction [MAS-PCR] for identification of the Hb J-Singapore and Hb J-Buda. The locations and orientations of primers (C1 and SP05, SP02 and B, and C1 and B) that produce fragments of 632, 455, and 975 bp specific for Hb J-Singapore, Hb J-Buda, and internal control fragment, respectively are indicated. Lanes 1 and 4: normal DNA controls, lane 2: Hb J-Singapore carrier (the propositus), lanes 3: Hb J-Buda carrier. M represents the GeneRuler 100 bp DNA ladder.

seconds, 65°C for 30 seconds, 72°C for two minutes plus an additional 20 seconds in every cycle. The amplified product was analyzed on 1.5% agarose gel electrophoresis and visualized under UV light after ethidium bromide staining. The 632 bp amplified fragment from Hb J-Singapore allele was observed in this case (Figure 3).

Discussion

In the present study, Hb J-Singapore presented only one mutation at codon 79 (Ala→Gly) of α_2-globin gene. Thus, the absence of DNA evidence for the α78 (Asn→Asp) substitution meant that the deamidation occurred at the protein level, which was favored by the adjacent glycine residue[3]. This deamidation was accountable for having more negative charge of Hb J-Singapore than HbA. Thus, it was eluted ahead of HbA with the retention time of 1.83 minutes on HPLC chromatogram and 100 to 110 seconds (zone 12) on CE electrophoregram[3]. The previous study also suggested that the substitution of glycine for alanine at position α79 did not alter the surface charge of Hb J-Singapore. Thus, it would be expected to co-elute with HbA[3]. Hb J-Singapore is non-pathological Hb variant and it is usually discovered during a systematic study performed within program for prevention of thalassemia. However, it may produce clinically relevant phenotypes when it co-inherits with α-thalassemia-1 or other α-globin gene variants. There have been only two cases reported for heterozygote of Hb J-Singapore[1,3], and no report for the combination of Hb J-Singapore and α-thalassemia-1. However, a carefully genetic counseling is needed for the Hb J-Singapore heterozygote who has a partner with α-thalassemia-1 trait since there is 25% chance of having a child with Hb J-Singapore and α-thalassemia-1 combination.

On CE electrophoregram, Hb J-Singapore presents in the same migration time (zone 12) of Hb J-Buda and Hb Bart's (γ4)[9]. The CE separates molecules on the basis of their charge and their hydrodynamic volume[10]. Therefore, Hb J-Singapore, Hb J-Buda, and Hb Bart's might have similar charge and hydrodynamic volume. In addition, the HPLC patterns of Hb J-Singapore and Hb J-Buda are similar. Thus, there are potentially pitfalls for misinterpretation. The MAS-PCR can, in the future, provide a rapid method for identification of these hemoglobinopathies.

In conclusion, although Hb J-Singapore is very rare, it can be found in Thai people. Therefore, knowledge and understanding of this Hb variant will be used to assist in diagnosis, management, and counseling for patients with this hemoglobinopathy.

What is already known on this topic?

Hb J-Singapore [α79(EF8)Ala→Gly, GCG>GGG] is a very rare α-globin chain variant. On capillary electrophoresis Hb J-Singapore presents in the same migration time (zone 12) of Hb J-Buda and Hb Bart's. There is no specific PCR analysis for detection of Hb J-Singapore.

What this study adds?

The authors reported, for the first time, the discovery of Hb J-Singapore in a Thai pregnant woman. In addition, the MAS-PCR was developed for differentiating of Hb J-Singapore and Hb J-Buda. Therefore, the better understanding of HPLC chromatogram and CE electrophoregram patterns and the developed MAS-PCR method is useful for genetic counseling, prevention, and control programs for thalassemia and hemoglobinopathy.

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Potential conflicts of interest

The authors declare no conflict of interest.

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