CASE REPORT

Siriraj I $^{6}\gamma^{(\gamma\delta\beta)}$-thalassaemia causing severe thalassaemia intermedia in compound heterozygous state with IVS1-1(G→T) mutation

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Abstract

The Siriraj I $^{6}\gamma^{(\gamma\delta\beta)}$-thalassaemia is a novel mutation involving a 118kb deletion of the β-globin gene cluster. It was first reported in 2012 in two unrelated families from the southern part of Thailand. The carriers in the heterozygous state are clinically asymptomatic. Nonetheless, its complex interaction with other β-thalassaemia could give rise to different clinical phenotypes, ranging from mild thalassaemia intermedia to thalassaemia major. We report here a case of a six-year-old Malay boy, presented with pallor, growth failure and hepatosplenomegaly. His haemoglobin at presentation was 9.2g/dL with a mean cell haemoglobin of 22.6pg and a mean cell volume of 69.9fl. His peripheral blood smear showed features of thalassaemia intermedia. Haemoglobin (Hb) analysis revealed markedly raised Hb F (83%), normal HbA₂ levels and absent HbA. Deoxyribonucleic acid (DNA) analysis showed compound heterozygous IVS1-1 (G→T) β-globin gene mutation and Siriraj I $^{6}\gamma^{(\gamma\delta\beta)}$-deletion (genotype $^{6}\gamma^{(\gamma\delta\beta)}$/ $^{6}\gamma^{(\gamma\delta\beta)}$ Siriraj I deletion). Both his father and elder sister are carriers of Siriraj I $^{6}\gamma^{(\gamma\delta\beta)}$-thalassaemia while his mother carries IVS1-1 (G→T) gene mutation. Clinically, the patient is transfusion dependent on six weekly regime. To the best of our knowledge, this is the first reported case in Malaysia involving unique Siriraj I $^{6}\gamma^{(\gamma\delta\beta)}$-thalassaemia and IVS1-1 (G→T) in a compound heterozygous state. In summary, detection of Siriraj I $^{6}\gamma^{(\gamma\delta\beta)}$-thalassaemia is essential as this deletion can lead to severe disease upon interaction with a β-thalassemia point mutation as demonstrated in our case. The establishment of effective carrier screening and genetic counselling is important to prevent its adverse consequences.

Keywords: Thalassaemia intermedia, Siriraj I $^{6}\gamma^{(\gamma\delta\beta)}$-thalassaemia, IVS 1-1 (G→T)

INTRODUCTION

β-thalassaemia is an autosomal haematological disorder resulting in genetic lesions which include all or part of the β-globin gene complex.¹ This inherited disorder was originally characteristic of the tropics and subtropics region but is now worldwide due to migratory fluxes from different countries and eventually present as a global public health problem.² It is estimated that approximately 1% to 5% of the global population carries thalassaemia mutations.³ In Malaysia, thalassaemia is the commonest single gene disorder with a carrier rate of 4.5% to 6%.⁴ The human β-globin gene cluster resides within 80 kilobases (kb) on the short arm of chromosome 11.⁵ This complex is structurally arranged and developmentally expressed in the order of $5^{‘}$-ε-γ-δ-β. The expression of these genes is regulated by the locus control region (LCR) that lies 20 kb upstream of the ε-globin gene.⁶ In the simplest situation of β-thalassaemia, only β-globin production is defective and other β-globin genes are normally produced. More complex entities involve a defective synthesis of more than one β-globin genes.

Co-inheritance of δβ thalassaemia and δβ thalassaemia could give rise to different clinical phenotypes, ranging from mild thalassaemia intermedia to thalassaemia major (Li et al. 2001).⁷ The molecular heterogeneity and its complex interaction are key elements in modifying the clinical severity. The establishment
of the diagnosis can be challenging. Both conventional method (gel electrophoresis, capillary electrophoresis or high-performance liquid chromatography) and advanced method (DNA analysis) are required. DNA analysis using multiplex gap polymerase chain reaction (Gap-PCR) and multiplex amplification refractory mutation system (MARMS) are useful in determining the genotypes. In this case report, we described a Malay patient with compound heterozygous of IVS1-1 (G→T) / Siriraj I x(γAδβ)δ-thalassaemia who presented with severe thalassaemia intermedia at six years of age.

CASE REPORT

A six-year-old Malay boy presented with moderate anaemia associated with growth failure in the year 2004. Previously, he had no known medical illness and had never been transfused before. Both parents were of Malay descent. However, his father’s ancestors were from Pattani, Thailand. Physical examination revealed thalassaemic facies (frontal bossing and maxillary expansion), short stature (height of 107cm and weight of 15.3kg) and hepatosplenomegaly. Examination of other systems was unremarkable.

His haemoglobin at presentation was 9.2g/dL with a mean cell haemoglobin of 22.6pg and a mean cell volume of 69.9fl (Table 1). Peripheral blood film showed hypochromic microcytic red blood cells and marked anisopikilocytosis with the presence of target cells, schistocytes, polychromatocytes, nucleated red blood cell and Howell-Jolly bodies. Dense band at the F region was visible in the alkaline cellulose acetate electrophoresis. Quantitation of haemoglobins by high-performance liquid chromatography (HPLC) revealed markedly raised HbF (83%), normal HbA2 levels and absent HbA. H inclusion study was negative. The Kleihauer test showed heterogeneous pattern of HbF positivity.

This prompted a more accurate DNA-based analysis. The multiplex amplification refractory mutation system (MARMS) and multiplex gap polymerase chain reaction (Gap-PCR) revealed

TABLE 1: Summary of the haematological findings in the family

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient</th>
<th>Father</th>
<th>Mother</th>
<th>Sister</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Full blood count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.2</td>
<td>14.4</td>
<td>12.3</td>
<td>13.9</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>69.9</td>
<td>74.6</td>
<td>67.6</td>
<td>77.8</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>22.6</td>
<td>23.8</td>
<td>21.8</td>
<td>25.2</td>
</tr>
<tr>
<td>RBC (x10^12/L)</td>
<td>4.06</td>
<td>6.05</td>
<td>5.64</td>
<td>5.53</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>28.1</td>
<td>15.9</td>
<td>15.4</td>
<td>14.7</td>
</tr>
<tr>
<td>Clinical phenotype</td>
<td>Severe Thalassaemia Intermedia</td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Hb Analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Cellulose Acetate electrophoresis</td>
<td>Dense band at F region</td>
<td>Dense band at F region</td>
<td>Prominent band at A_2 region</td>
<td>Dense band at F region</td>
</tr>
<tr>
<td>HPLC</td>
<td>Markedly raised Hb F (83%) with normal Hb A_2 (2.4%).</td>
<td>Raised Hb F (29.4%) with normal Hb A_2 (2%).</td>
<td>Raised HbA_2 (5.5%) with mildly raised Hb F (1.2%).</td>
<td>Raised Hb F (30.7%) with normal Hb A_2 (2%).</td>
</tr>
<tr>
<td>Kleihauer test</td>
<td>Heterogenous positivity pattern</td>
<td>Heterogenous positivity pattern</td>
<td>-</td>
<td>Heterogenous positivity pattern</td>
</tr>
<tr>
<td>Genotype</td>
<td>β^&lt;IVS1-1/β^ Siriraj I deletion</td>
<td>β^N/β^ Siriraj I deletion</td>
<td>β^N/β^ IVS1-1</td>
<td>β^N/β^ Siriraj I deletion</td>
</tr>
</tbody>
</table>
compound heterozygous IVS1-1 (G→T) β-globin gene mutation and Siriraj I γ(γδβ)-thalassaemia (genotype βIVS1-1/βSiriraj I deletion) (Fig. 1 and 2). The β gene sequencing also showed a heterozygous point mutation of G→T at IVS-1 position 1 in the β-globin gene (Fig. 3).

Family studies revealed that his parents and elder sister were clinically asymptomatic with no hepatosplenomegaly was noted. The summary of the haematological findings in the family are shown in Table 1 and the DNA tests results are shown in Figures 1-3.

The patient is currently a university student, who is socially active. Unfortunately, he was transplant-ineligible as there was no HLA-matched donor found. At present, he requires six weekly blood transfusions to maintain haemoglobin level at 9 to 10 g/dL. The disease is complicated with chronic iron overload which needs iron chelation therapy and multiple endocrinopathies including impaired glucose tolerance and partial cortisone deficiency.
DISCUSSION

The Siriraj I G γ(γδβ)0-thalassaemia is a mutation involving a 118kb deletion of the β-globin gene cluster. The deletion removes 118kb of DNA between 5′ γ-globin gene and 3′ L1 repetitive sequences, resulting in the presence of 620bp fragments in multiplex gap-PCR. It was first reported in 2012 in two unrelated families from the southern part of Thailand. Interestingly, the ancestors of the family in our case migrated from Pattani which was a town in the far south of Thailand. As observed in this case, the heterozygotes clinically have thalassaemia minor with normal Hb levels and mild hypochromic microcytic changes in the red cells.

The synthesis of foetal haemoglobin (HbF) is normally less than 0.6% of the total haemoglobin in adults. Hereditary persistent fetal haemoglobin (HPFH) and δβ-thalassaemia are both characterised by a high level of HbF production into adulthood. The distinction between these conditions is subtle. HPFH heterozygotes usually have higher levels of HbF (up to 30%) with normal red blood cell indices and pancellular distribution of F-cells in the Kleihauer test. Whereas, heterozygotes for δβ-thalassaemia have a modest increase of HbF (5–20%) with hypochromic microcytic red cell and heterocellular distribution of F-cells in Kleihauer test.

The molecular characterisation is crucial for differential diagnosis as compound heterozygosity of HPFH and β thalassaemia behaves clinically as thalassaemia minor in opposition to co-inheritance of δβ thalassaemia and β thalassaemia which may give rise to β thalassaemia major. In our case, the heterozygous father and sister have raised HbF level (29.4% and 30.7% respectively) with a heterocellular distribution of F-cells and hypochromic microcytic red blood cells.

In Malaysia, the IVS1-1 (G→T) β-globin gene mutation is one of the common β-thalassaemia mutations among Malay population. Others include HbE [Cd 26 (G→A)] and IVS1-1 (G→T) β-globin gene mutations. These three types of mutations are found in more than 75% of β-thalassaemia carriers. The mutation at IVS1-1 causes abnormal splicing of RNA due to the single G→T base-pair substitution in the splice junction of the β-globin gene. The normal splicing of RNA is completely abolished and thus results in the β0-thalassaemia phenotype among the homozygotes. In a heterozygote state, an individual with IVS1-1 (G→T) β-globin gene mutation is clinically asymptomatic, as in the case of the patient’s mother (genotype β(0/βIVS1-1). Compound heterozygosity of IVS1-1 (G→T)/ Siriraj I G γ(γδβ)0-thalassaemia results severe β-thalassaemia intermedia phenotype. The patient in this report had significantly high level of HbF (83%) with a heterocellular distribution of red cells in the Kleihauer test. Regular blood transfusion was started at the age of six for moderate anaemia with growth failure. He required six weekly blood transfusions then on to maintain a mean haemoglobin level of above 10 g/dL for normal growth and development. In addition, iron chelation therapy was initiated to prevent iron overloading. Li et al. described two cases of β/δβ thalassaemia in China. According to the study, co-inheritance of β thalassaemia and δβ thalassaemia gives rise to different clinical phenotypes, ranging from mild thalassaemia intermedia to thalassaemia major in relation to several factors. For instances, the nature of β-thalassaemia mutation, the range of gene deletion, the coexisting α-thalassaemia, and other genetic factors that can elevate γ-chain production. Siriraj I G γ(γδβ)0-thalassaemia involves a long-segment deletion of 118 kb DNA.
between 5′-γ-globin gene and 3’ L1 repetitive sequences. Therefore, compound heterozygosity of this defect with β-thalassaemia mutation may result in severe transfusion-dependent β-thalassaemia as illustrated in our case.

In Malaysia, this is the first reported case involving unique Siriraj I γ(γδβ)0-thalassaemia and IVS1-1 (G→T) in a compound heterozygous state. The exact incidence of Siriraj I γ(γδβ)0-thalassaemia in Malaysia remains unknown. The establishment of the diagnosis is challenging and labourious. Confirmation can only be made by molecular analysis. For characterisation of rare and unknown β-globin gene cluster deletions, the most widely used technique is gap polymerase chain reaction (gap-PCR) as optimised by Tritipsombut et al. suitable for South-East Asian countries to provide a relatively quick, accurate and cost-effective diagnostic test for β-thalassaemia and deletion types of HPFH that can be applied in any molecular diagnostic laboratory equipped with standard PCR apparatus. As in this case, Siriraj I γ(γδβ)0-thalassaemia deletion was identified by the presence of specific 620bp fragments in multiplex gap-PCR (Fig.2). In Malaysia, Hassan et al. from the Institute for Medical Research (IMR) designed a PCR approach to characterise the spectrum of β-globin gene mutations among Malaysians. They modified the multiplex amplification refractory mutation system (MARMS) developed by Bhardwaj et al. by adding primers according to the local mutation heterogeneity. A total of 20 β-globin gene mutations were able to be concurrently analysed in five sets of MARMS and an additional ARMS test, which in our case resulted in the identification of the mutations in this family.

CONCLUSION

In summary, detection of Siriraj I γ(γδβ)0-thalassaemia is requisite as this deletion can lead to severe disease upon interaction with a β-thalassaemia point mutation as demonstrated in our case. The knowledge gained in this case study is useful in the development of effective carrier screening and genetic counselling for the future generation.

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Conflict of interest: The authors declare no conflict of interest.

REFERENCES


