Hemoglobin Ottawa (HBA2:c.46G>C) and β+ thalassemia (HBB:c.-138C>T) detected in an Indian male by capillary zone electrophoresis

Beverley M. Pullon,1 Jordyn A. Moore2
1Hematology Department, Waikato Hospital, Hamilton; 2Specialist Biochemistry, Canterbury Health Laboratories, Christchurch, New Zealand

Abstract

Hemoglobin (Hb) Ottawa [α15(A13)Gly>Arg], also known as Hb Siam, results from GGT→CGT mutation in codon 15 of either HBA1 or HBA2. Hb Ottawa carriers typically have normal hematology but when the variant is co-inherited with either α or β thalassemia, microcytic red cell indices were observed. The percentage of variant detected using routine methodology was variable (14-33%), with a higher percentage found when co-inherited with an abnormal α-globin genotype. The case presented here involved an Indian male with microcytic red cell indices, who was heterozygous for Hb Ottawa (HBA2:c.46G>C) and β+ thalassemia (HBB:c.-138C>T). This case represents the first reported finding of Hb Ottawa in the Indian population, as well as the first time capillary zone electrophoresis (CZE) has been used to identify the variant. The abnormal red cell indices were attributed to co-inheritance of β+ thalassemia mutation (HBB:c.-138C>T), which alters binding of transcriptional factors to the HBB promoter and reduces transcription from the allele. The mild β+ thalassemia mutation has commonly been found in the Indian population.

Introduction

Hb Ottawa [α15(A13)Gly>Arg] was first identified in 1974 in an 82-year-old Canadian male of Polish descent, who presented with mild anemia.1 The same year, a similar variant was reported in a healthy 28-year-old Thai individual.2 However, microcytic red cell indices have previously been described in two cases due to co-inheritance with α-thalassemia-1 and β+ thalassemia.3

The β+ thalassemia mutation (HBB:c.-138C>T) was first described in 198411 and has frequently been observed in the American black population.12 This mutation occurs in the promoter region of the HBB gene and impairs binding of transcriptional factors to the promoter, reducing transcription and giving rise to β+ thalassemia.11 Typically the mutation is associated with mild hypochromic microcytosis and elevated levels of HbA2 and HbF.12-13

This paper describes a case of double heterozygosity for Hb Ottawa (HBA2:c.46G>C) and β+ thalassemia (HBB:c.-138C>T) in a 47-year-old Indian male detected using CZE.

Case Report

A hemoglobinopathy/thalassemia screen was requested for the subject of this investigation after he was noted to have thalassemic carrier red blood cell indices. His full blood count, analyzed on an electronic Sysmex XN900 (Sysmex Corporation, Kobe, Japan), showed a normal Hb level of 148 g/L (NR = 130-175), with reduced mean cell volume (MCV) 71 fL (NR = 80-99) and mean cell Hb (MCH) 23 pg (NR = 27-33). His white cell and platelet count were normal. The serum ferritin level, which was requested for the subject of this investigation, showed a normal level of 120 ng/mL (NR = 20-90).

CZE was performed on a Sebia Capillaries 2 Flex Piercing analyzer (Sebia, Lisses, France) using the HbE programme and showed a normal HbF level of <1% (NR = <1%), with raised HbA2 of 5.7% (NR = 2.2-3.3%) suggesting an underlying β thalassemia. Two aberrant peaks were identified on the chromatogram; a major peak eluting in zone S (x-axis 214) constituting 26%, and a minor peak eluting in zone 1 (x-axis 276) constituting 1.4% (Figure 1). The association of the major peak with a corresponding slow HbA2 peak (minor peak) suggested the presence of a variant α-globin species. The presence of an α-globin variant meant the peak representing HbA2 (4.3%) and variant HbA2 (1.4%) should be added together to reflect the total HbA2 level of 5.7%.14

Confirmatory testing was carried out using cation exchange HPLC on a Bio-Rad D10 instrument, (Bio-Rad Laboratories, Hercules, CA, USA) using the HbA2/F extended programme. The result showed a normal HbF level of 0.8% (NR = <1%), with raised HbA2 of 5.0% (NR = 2.2-3.3%). Hb Ottawa appeared as an abnormal peak of 23.2% eluting in the HbS window (4.02-4.30) at retention time 4.16 seconds.

α Thalassemia testing performed using the immunochromatographic (IC) strip test for α thalassemia (+Med Laboratories, Bangkok, Thailand) was negative. Alkaline Hb Ep (cellulose acetate pH 8.5) and acid Hb Ep (citrate agar pH 5.9) were achieved using the Sebia Hydragel (E) and Sebia Hydragel (A) systems.
Hydragel Acid (E) Hb kits respectively (Sebia, Lisses, France). Ep strips were stained with amidoblack. Alkaline Ep revealed the presence of two major bands. The mobility of the bands was consistent with that of HbA and a variant migrating in the HbS/D position. Acid Ep showed the variant to have mobility associated with HbD. An in-house sickle solubility test using sodium dithionite was negative and the isopropanol flocculation test for an unstable hemoglobin was normal.

DNA was extracted from peripheral blood and studies for mutation in HBA1, HBA2 and HBB undertaken. Mutation analysis was determined by direct sequencing of overlapping polymerase chain reaction (PCR) products spanning the entire α- and β-globin genes. Sanger sequencing methodology was performed and the products separated by capillary electrophoresis on an ABI3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequencing indicated the subject was heterozygous for Hb Ottawa (HBA2:c.46G>C) on the α2-globin gene and heterozygous for β+ thalassemia mutation (HBB:c.-138C>T) within the promoter region of the β-globin gene. A summary of relevant results for the subject is provided in Table 1.

**Discussion**

Hb Ottawa has been reported in several ethnic groups to date including Canadian–Polish,1 Chinese,2,5,7,10 Thai,6,8 African9 and for the first time, described here in an Indian male residing within New Zealand. In addition this is the first reported case of Hb Ottawa discovered using CZE, with the variant found to elute in zone S. The identification of the variant as Hb Ottawa was subsequently confirmed by DNA sequencing. Previously described heterozygotes for Hb Ottawa did not present with any clinical symptoms or hematological changes.2,5,7,9 The original Canadian-Polish patient had mild anemia due to iron and folate deficiency, together with a chronic uremic state.1 It was reported unlikely the subject’s anemia was contributed to by Hb Ottawa and this was supported by the findings from later cases.2,5,7,9

The level of Hb Ottawa detected for our

<table>
<thead>
<tr>
<th>Reference</th>
<th>Ethnicity</th>
<th>MCV fl (80-99)</th>
<th>Hb Ottawa %</th>
<th>Affected α-globin gene</th>
<th>Co-inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>This Case</td>
<td>Indian</td>
<td>71</td>
<td>26</td>
<td>α2</td>
<td>β+ thalassemia</td>
</tr>
<tr>
<td>Vella et al.1</td>
<td>Polish-Canadian</td>
<td>80</td>
<td>25</td>
<td>NA</td>
<td>HBB:c.-138C&gt;T</td>
</tr>
<tr>
<td>Pootrakul et al.2</td>
<td>Chinese</td>
<td>Normal</td>
<td>15</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Gu et al.3</td>
<td>Chinese</td>
<td>Normal</td>
<td>16</td>
<td>α1</td>
<td></td>
</tr>
<tr>
<td>Yodsonnet et al.4</td>
<td>Thai</td>
<td>64</td>
<td>33</td>
<td>α1</td>
<td>α-thalassemia-1</td>
</tr>
<tr>
<td>Turbpiboon et al.8</td>
<td>Thai</td>
<td>64</td>
<td>17</td>
<td>α1</td>
<td>β+ thalassemia</td>
</tr>
<tr>
<td>Huang et al.5</td>
<td>Chinese</td>
<td>87</td>
<td>14</td>
<td>α2</td>
<td>HBB:c.52A&gt;T</td>
</tr>
<tr>
<td>Silva et al.9</td>
<td>African</td>
<td>77</td>
<td>23</td>
<td>α1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>%</th>
<th>Normal Values %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>68.3</td>
<td></td>
</tr>
<tr>
<td>Hb S zone</td>
<td>26.0</td>
<td></td>
</tr>
<tr>
<td>Hb A2</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Zone 1</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Capillarys hemoglobin (E) electrophoresis chromatogram showing aberrant peaks in zone S (26%) and zone 1 (1.4%), which correspond to Hb Ottawa and the associated variant HbA2 peak respectively. The total level of HbA2 was 5.7%.

**Table 1. Summary of data for Hb Ottawa.**
subject (26%) was similar to that observed in the first case reported by Vella et al.\textsuperscript{1} (25%) and two cases by Silva et al.\textsuperscript{9} (23% and 24%) but was higher than in some other reports; 14%,\textsuperscript{3} 15%,\textsuperscript{2,5} 16%,\textsuperscript{2,5} 17%\textsuperscript{8} (Table 1). The variation may be attributed to differences in methodology for variant quantitation. Other factors to consider include co-inherited α or β thalassemia. None of the above cases had an abnormal α-globin genotype. However, the 21-year-old Thai female reported by Yodsowan et al.\textsuperscript{9} had 33% Hb Ottawa. The relatively elevated percentage of variant, along with observed microcytosis for this case, could be attributed to co-inheritance of an abnormal α-globin genotype (α-thalassemia-1). With regard to co-inheritance of β thalassemia, the level of Hb Ottawa detected in association with β\textsuperscript{+} thalassemia for the proband reported by Turbpaiboon et al.\textsuperscript{8} was similar to that of the subject’s father and sister, who did not have β thalassemia. It seems likely therefore, that even under conditions of reduced β-globin chain production, there was no decrease in the combination of α- and β-globin chains for Hb Ottawa. This would be consistent with the proposed mechanism of αβ dimer assembly, which is thought to occur via electrostatic interaction between the positively charged α-globin and negatively charged β-globin chain.\textsuperscript{8} For Hb Ottawa the Gly to Arg substitution promotes interaction with β-globin chains for Hb Ottawa. This would result in the mild thalassemia presentation. It should be noted that Hb instability was not demonstrated for our subject during this investigation but one previous report suggested Hb Ottawa was mildly unstable.\textsuperscript{8} Stability for Hb Ottawa was only mentioned in one other literature report that described stability as normal.\textsuperscript{3} Regardless, many reports of unstable Hb variants indicate normal MCV in the absence of complicating factors.\textsuperscript{16}

The β\textsuperscript{+} thalassemia mutation (HBB:c.-138C>T) was identified as African in origin by Orkin et al.,\textsuperscript{11} but has also been observed in Asian Indians with the same nucleotide substitution but on a different chromosomal background as indicated by haplotype analysis, suggesting two independent mutation origins.\textsuperscript{17} Even though the β\textsuperscript{+} thalassemia allele is mild, carriers of the HBB promoter mutation tend to have relatively high values for HbA\textsubscript{2}\textsuperscript{12-13} but with variable HbF levels that might be reflective of the ethnic background and haplotype of the individual.\textsuperscript{18}

The level of HbA\textsubscript{2} detected in our subject was 5.7%, which was within the range observed by Huisman\textsuperscript{13} (5.35-5.95%) and slightly higher than that reported by Gonzalez et al.\textsuperscript{15} High HbA\textsubscript{2} levels associated with HBB:c.-138C>T have been suggested to be caused by reduced binding of transcriptional factors to the HBB promoter and indirect enhancement of HBD transcription.\textsuperscript{13,14} This mechanism is in addition to the commonly accepted hypothesis that reduced β-globin production results in excess α-globin available for formation of HbA\textsubscript{2}. In contrast, HbF of <1% for our subject was much lower than the 2-4% observed by both Huisman\textsuperscript{13} and Gonzalez et al.\textsuperscript{15} One of the difficulties in comparing results across laboratories is diverse methodology used to assess the level of HbF, which can have variable sensitivity, particularly at low levels.\textsuperscript{15} However, for our subject HbF <1% was detected by both CZE and HPLC methods. For the cases described by both Huisman\textsuperscript{13} and Gonzalez et al.\textsuperscript{15} the individuals were of American Black ethnicity, so a different chromosomal background could explain the lower levels observed in our Indian subject.\textsuperscript{15} Factors such as these make it difficult to be dogmatic about the typical HbA\textsubscript{2}, HbF and red cell indices associated with a particular allele, particularly in view of the paucity of data available.

Conclusions

We present here the first report of compound heterozygosity for Hb Ottawa (HBA\textsubscript{1}:c.46G>C and HBB:c.-138C>T) in a person of Indian ethnicity. The Hb variant was discovered using CZE where elution of the variant species occurred in zone S. Hb Ottawa results from GGT>CCT mutation in codon 15 of either the HBA\textsubscript{1} or HBA\textsubscript{2} globin genes. Hb Ottawa carriers typically have a normal hematological profile but if co-inherited
with either α or β thalassemia have microcytic red cell indices, as was the case for our subject due to co-inheritance of β′ thalassemia mutation (HBB:c.-138C>T). The percentage of Hb Ottawa detected is variable, with higher percentages found when the variant is co-inherited with an abnormal α-globin genotype. It was difficult to determine whether the α-globin gene the mutation was on contributed to variation in the variant quantity detected amongst reports. However, it seems likely different methodologies would be a factor and thus it would be important to be aware of potential variation in levels detected using particular methods when considering the possibility of co-inherited thalassemia for this mutation.

References