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

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Abstract

Hemoglobin (Hb) Ottawa [α15(A13)Gly>Arg], also known as Hb Siam, results from *G*GT>*C*GT 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 second occurrence of the variant was reported in a healthy 28-year-old Thai individual of Chinese ancestry and referred to as Hb Siam.[2] According to the Hb variant (http://globin.cse.psu.edu/hbvar/menu.html)[3] and ithanet (https://www.ithanet.eu/)[4] databases, Hb Ottawa has been found in individuals of Canadian-Polish, Chinese, Thai and African ethnicity but has not been reported in the Indian population before. The quantity of Hb Ottawa found in heterozygotes has been shown to vary between 14%[5] and 33%.[6] Most patients heterozygous for the mutation have normal clinical presentation and were diagnosed during thalassemia screening by Hb electrophoresis (Ep), or cation exchange High Performance Liquid Chromatography (HPLC) when an abnormal Hb species was identified.

Hb Ottawa results from *G*GT>*C*GT mutation in codon 15 of either *HBA1*[5-6,8-9] or *HBA2*[5] and has been reported as a mildly unstable Hb variant.[8] Hb Ottawa is not known to have an α-thalassemic effect, so typically the variant is associated with normal hematology in the heterozygote.[1,2,5,6,9,10] However, microcytic red cell indices have previously been described in two cases due to co-inheritance with α-thalassemia-1[6] and β^0 thalassemia.[8]

The β$^+$ thalassemia mutation (*HBB*:c.-138C>T) was first described in 1984[11] and has frequently been observed in the American black population.[12] This mutation occurs in the promotor region of the *HBB* gene and impairs binding of transcriptional factors to the promotor, reducing transcription and giving rise to β$^+$ thalassemia.[11] Typically the mutation is associated with mild hypochromic microcytosis and elevated levels of HbA_2 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 analyzed on a Cobas 6000 instrument (Roche Diagnostics, Indianapolis, IN, USA) was normal at 89 ng/mL (NR = 20-90).

CZE was performed on a Sebia Capillarys 2 Flex Piercing analyzer (Sebia, Lisses, France) using the HbE programme and showed a normal HbF level of <1% (NR = <1%), with raised HbA_2 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 HbA_2 peak (minor peak) suggested the presence of a variant α-globin species. The presence of an α-globin variant meant the peak representing HbA_2 (4.3%) and variant HbA_2 (1.4%) should be added together to reflect the total HbA_2 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 HbA_2/F extended programme. The result showed a normal HbF level of 0.8% (NR = <1%), with raised HbA_2 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 (i+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

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Key words: Hb Ottawa; β$^+$ thalassemia; CZE; PCR.

Acknowledgments: PathLab Waikato for their assistance and LabPLUS Auckland for molecular testing.

Contributions: BMP, conception, design, drafting and final approval of manuscript; analysis and interpretation of hematology data and CZE results; provided Figure 1 and Table 1. JAM, provided interpretation of molecular results; critically revised manuscript.

Conflict of interests: The authors declare no potential conflict of interests.

Received for publication: 4 December 2019.
Revision received: 23 April 2020.
Accepted for publication: 27 April 2020.

Thalassemia Reports 2020; 10:8733
doi:10.4081/thal.2020.8733

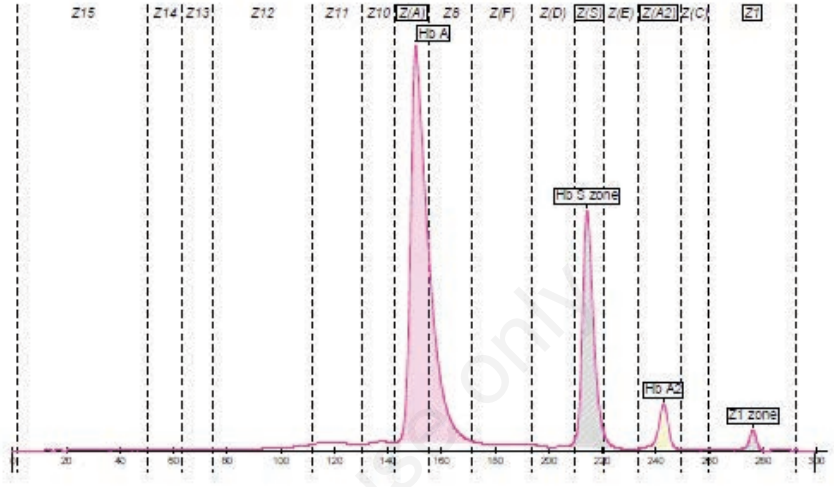
Hydragel Acid (E) Hb kits respectively (Sebia, Lisses, France). Ep strips were stained with amidoblack. Alkaline Ep revealed the presence of two major bands. The mobility of the bands was consistent with that of HbA and a variant migrating in the HbS/D position. Acid Ep showed the variant to have mobility 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 promotor 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] African[9] 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



Name	%	Normal Values %
Hb A	68.3	
Hb S zone	26.0	
Hb A2	4.3	
Z1 zone	1.4	

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 HbA_2 peak respectively. The total level of HbA_2 was 5.7%.

Table 1. Summary of data for Hb Ottawa.

Reference	Ethnicity	MCV fL (80-99)	Hb Ottawa %	Affected α-globin gene	Co-inheritance
This Case	Indian	71	26	α2	β+ thalassemia *HBB*:c.-138C>T
Vella *et al.*,[1]	Polish-Canadian	80	25	NA	
Pootrakul *et al.*[2]	Chinese	Normal	15	NA	
Gu *et al.*[7]	Chinese	Normal	16	α1	
Yodsowan*et al.*[6]	Thai	64	33	α1	α-thalassemia-1
Turbpaiboon *et al.*[8]	Thai	64	17	α1	β^0 thalassemia *HBB*:c.52A>T
		90	17	α1	
		76	17	α1	
Huang *et al.*[5]	Chinese	87	14	α2	
		89	15		
		87	16		
		84	17		
Silva *et al.*[9]	African	77	23	α1	
		NA	24		

MCV, mean cell volume; NA, not available.

subject (26%) was similar to that observed in the first case reported by Vella *et al.*[1] (25%) and two cases by Silva *et al.*[9] (23% and 24%) but was higher than in some other reports; 14%,[5] 15%,[2,5] 16%,[5,7] 17%[5,8] (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.*[6] 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^0 thalassemia for the proband reported by Turbpaiboon *et al.*[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.[8] For Hb Ottawa the Gly to Arg substitution produces a more positively charged α-globin chain, maintaining the electrostatic interaction required to facilitate αβ dimerization.[8] This indicates Hb Ottawa does not have an α-thalassemic effect arising from altered affinity of Hb Ottawa for β-globin chains during tetramer assembly.

It has also been suggested that the percentage of Hb Ottawa might be determined by whether the nucleotide substitution *G*GT>*C*GT is on the *HBA1* or *HBA2* gene.[6,8] In persons with four normal α-globin alleles, there is a higher ratio of mRNA transcription of the *HBA2* gene compared to *HBA1* (-2.75:1).[12] However, there is higher translation efficiency of *HBA1*, resulting in almost equal synthesis of α-globin chains from either the *HBA1* or *HBA2* genes.[15] The presence of a mildly unstable α-globin chain variant, such as Hb Ottawa, could influence the α2:α1 ratio observed at the protein level. For a heterozygote carrier of a stable α-globin chain variant, slightly lower levels of variant might be expected if the mutation was on the *HBA1* gene, as the ratio of α2-mRNA to α1-mRNA is about 1.2:1 at the protein level.[15] However, minor instability of Hb Ottawa may affect the percentage of variant detected.

Variant levels of approximately 17% have been found when the mutation occurs on either the *HBA1*[7-8] or *HBA2* gene.[5] Similarly, approximately 25% has been seen with mutation of either globin gene as reported by Silva *et al.*[9] and for the present case. In the earlier studies of Vella *et al.*[1] and Pootrakul *et al.*,[2] no information was available regarding which gene the mutation was described in (Table 1).[1,2,5-9] Complicating matters when an α-globin chain variant is co-inherited with α thalassemia the α2-mRNA to α1-mRNA ratio can increase (up from 1.2:1 to -2:1). This would likely have contributed to the increase in variant level detected by Yodsowan *et al.*,[6] whose propositus had 33% Hb Ottawa, due to co-inheritance of an α-thalassemia-1 genotype, with only two functional α-globin alleles.[6]

For the present case, and that of two Thai females[6,8] microcytic indices (MCV of 71, 64 and 64 fL respectively) were observed. All other heterozygote cases for Hb Ottawa had MCV within the normal range. The case reported here was a double heterozygote for Hb Ottawa and β^+ thalassemia (*HBB*:c.-138C>T). The β^+ thalassemia mutation is known to occur in the Indian population[4] and the resultant hypochromia and microcytosis, with MCV values of 70-74 fL are a well-known clinical finding.[3,12,13] In the case of Yodsowan *et al.*,[7] hypochromia and microcytosis were caused by compound heterozygosity for Hb Ottawa and α-thalassemia-1, while the case reported by Turbpaiboon *et al.*[8] was a double heterozygote for Hb Ottawa and β^o thalassemia mutation (*HBB*:c.52A>T). The sister of the proband described by Turbpaiboon *et al.*[8] was a heterozygote for Hb Ottawa alone, with an MCV of 76 fL. The slightly reduced MCV was attributed to iron deficiency in this case. One of the subjects reported by Silva *et al.*[9] had an MCV of 77 fL, but this was within the normal range (70-85 fL) for a 6-month-old child (Table 1).

It has been suggested that the MCV may be affected by the presence of a mildly unstable Hb, with some unstable Hbs in association with β thalassemia resulting in red cell indices consistent with thalassemia intermedia, or even thalassemia major.[16] However, the MCV for our subject was consistent with previous reports of simple heterozygotes for *HBB*:c.-138C>T, so it seems unlikely co-inheritance of Hb Ottawa has influenced 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.[8] Stability for Hb Ottawa was only mentioned in one other literature report that described stability as normal.[5] Regardless, many reports of unstable Hb variants indicate normal MCV in the absence of complicating factors.[16]

The β^+ thalassemia mutation (*HBB*:c.-138C>T) was identified as African in origin by Orkin *et al.*,[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.[17] Even though the β^+ thalassemia allele is mild, carriers of the *HBB* promoter mutation tend to have relatively high values for HbA_2[12-13] but with variable HbF levels that might be reflective of the ethnic background and haplotype of the individual.[18]

The level of HbA_2 detected in our subject was 5.7%, which was within the range observed by Huisman[13] (5.35-5.95%) and slightly higher than that reported by Gonzalez *et al.*[12] High HbA_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.[13,19] 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_2. In contrast, HbF of <1% for our subject was much lower than the 2-4% observed by both Huisman[13] and Gonzalez *et al.*[12] 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.[19] However, for our subject HbF <1% was detected by both CZE and HPLC methods. For the cases described by both Huisman[13] and Gonzalez *et al.*[12] the individuals were of American Black ethnicity, so a different chromosomal background could explain the lower levels observed in our Indian subject.[18] Factors such as these make it difficult to be dogmatic about the typical HbA_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 (*HBA2*:c.46G>C) and β^+ thalassemia (*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 *G*GT>*C*GT mutation in codon 15 of either the *HBA1* or *HBA2* 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.

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