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Coexistence of hemoglobin Handsworth and alpha 3.7 kb deletion in Caucasian woman in Poland

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Edyta Klimczak-Jajor[1]*, Joanna Skulimowska[1], Anna Ejduk[2], Katarzyna Guz[1], Małgorzata Uhrynowska[1], Ewa Brojer[1]

[1] Institute of Hematology and Transfusion Medicine, Department of Immunohematology and Immunology of Transfusion Medicine, Warsaw, Poland
[2] Institute of Hematology and Transfusion Medicine, Department of Hematology, Warsaw, Poland

Abstract

Background: This report presents a case of an adult Polish women of Caucasian origin who was heterozygous for the nondeletional mutation: Hb Handsworth (HBA2 or HBA1: c.55G > C, p.Gly19Arg) and deletional (-α$^{3.7}$) α-thalassemia mutation. Methods: The HbA_2 and HbF levels were measured by microcolumn chromatography and alkaline denaturation procedure, respectively, while electrophoresis was used to detect pathological hemoglobin fraction. The β- and α-globin genotypes were determined by DNA sequencing, gap-polymerase chain reaction, α gene triplication and MLPA. Results: The HbA_2 and HbF levels were normal, but hemoglobin electrophoresis on agarose gel alkaline pH showed a strong band migration in a position of hemoglobin S and faint bands in the neighborhood of band A on acid electrophoresis. Molecular analysis of the alpha globin cluster detected a point mutation at codon 19 in *HBA2* (c.55G > C, p.Gl- y19Arg) and deletion -α$^{3.7}$. Conclusions: Our compound heterozygosity does not produce severe clinical or hematological symptoms but it is important to say that in our part of Europe such cases do appear. Molecular analysis of the alpha globin cluster is required for correct diagnosis in patients with normal HbA_2 levels. Compound heterozygosity was unmasked by molecular diagnosis only.

Keywords:
α-thalassemia, Hb Handsworth, Alpha 3.7 kb deletion

Introduction

Inherited hemoglobinopathies are a large group of disorders that include thalassemia syndromes and structural hemoglobin (Hb) variants. Alpha thalassemia (α thalassemia) is caused by a decreased or abnormal synthesis of α globin chains. The genetic background includes deletions of one (α$^+$-thalassemia) or both α-globin genes (α^0-thalassemia) from the chromosome 16p13.3. Molecular analysis has shown that healthy individuals have genotype αα/αα (4 functional genes). The deletions which remove a single gene result in the silent carrier state (α-/αα), and others which remove two, three or four genes – lead to α-thalassmia trait (--/αα), HBH (hemoglobin H) disease (--/-α) or to Hb Barts hydrops fetalis syndrome respectively. The nondeletional mutations (point mutations or oligonucleotide insertions/deletions) have been recognized occasionally. Some of them may lead to more severe reduction in α-chain synthesis than the deletion α$^+$ -thalassemia [1] whereas others lead to abnormal α chain hemoglobins (α globin chain variants) which are non pathological [2, 3]. In this report we present the compound heterozygosity for nondeletional (Hb Handsworth) and deletional (-α$^{3.7}$) α-thalassemia mutations in an adult Polish woman. The 3.7 single-gene deletion (rightward deletion) is the most common alpha-thalassemia mutation worldwide [4] and in Poland [5, 6]. It is caused by reciprocal (inverse) recombination between Z segments (in α –globin gene cluster) during meiosis whereas the, Hb Handsworth (HBA2 or HBA1: c.55G > C, p.Gly19Arg) is rare and has never been detected in the Polish population. The first case of Hb Handsworth was determined in a 12 year old boy of West Indian origin [7]. It was also detected in a British patient [8], an Iran patient [3] and an inhabitant of the Netherlands [9].

In HPLC (high-performance liquid chromatography) analysis as well as in other routine laboratory methods the hemoglobin coded by DNA with this mutation, can be mistaken with HbS since both elute in the "S" window compartment [10].

The aim of this communication is to highlight the occurrence of compound hetreozygotes in α-globin gene defects in Northern Europe.

Materials and methods

A 32-year-old Polish woman was diagnosed for red blood cell microcytosis with no iron deficiency in the outpatient clinic of Institute of Hematology and Transfusion Medicine in Warsaw. Complete blood counts on Cell Dyn 4000 automated analyzer was performed within 24 hours of sample collection.

Biochemical analysis included microcolumn chromatography for quantization of HbA_2 (Beta-Thal HbA_2 Quick Column (Helena Biosciences)) and alkaline denaturation procedure for measurement of HbF and alkaline and acid hemoglobin electrophoresis (Sebia) to detect pathological hemoglobin fraction.

Genomic DNA was extracted from peripheral blood with EDTA leucocytes by using a blood genomic extraction kit Nucleospin Dx Blood (Machery Nagel) and kept at +4ºC. DNA concentration was determined by NANODROP spectrophotometer. The α-globin gene mutations were identified using the gap-polymerase reaction (gap-PCR) technique for the seven common deletions: single gene deletions (-α$^{3.7}$ -α$^{4.2}$) and both gene deletions: --^{FIL}, --^{SEA}, --$^{MED\ I}$, --$^{20.5}$, --^{THAI} as described by Kidd et al. [11] and α gene triplications using the

* Corresponding author: Edyta Klimczak-Jajor, Institute of Hematology and Transfusion Medicine, Indiry Gandhi 14, 02-776 Warsaw, Poland, Department of Immunohematology and Immunology of Transfusion Medicine, Chocimska 5, 00-957 Warsaw, Poland, Tel. 22 34 96 679 (673), fax 22 34 96 611, e-mail: eklimczak@ihit.waw.pl and edytaklimczakjajor@gmail.com and jskulimowska@ihit.waw.pl

PCR [12]. Results were visualized by separating PCR products using ethidium bromide-stained 2% agarose gel electrophoresis.

Multiplex ligation-dependent probe amplification (MLPA) assay in α-globin gene cluster was performed using the Salsa MLPA P140-C1 HBA kit (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions.

DNA sequencing of *HBA2*, *HBA1* [13] and *HBB* was performed using specific F and R primer set and the ABI Prism Big Dye Terminator v1.1 Cycle Sequencing kit on an ABI PRISM 3730 Genetic Analyser (Applied Biosystems, USA) according to the instructions of the manufacturer.

Results

Hematological analysis revealed: Hb 12,5 g/dl, MCV 72,6 fl and MCH 24,8 pg. Plasma ferritin concentration, serum bilirubin level and reticulocyte count were within normal. The levels of HbA_2 and HbF were 2,2% and 1,0% respectively. The detail hematological and biochemical profile is summarized in table I.

Hemoglobin electrophoresis on agarose gel alkaline pH showed a strong band migrating in a position of hemoglobin S and faint bands in the neighborhood of band A on acid electrophoresis were observed (Fig. 1).

Direct sequencing of the β-globin gene showed no mutations that might lead to pathological changes in the protein and direct sequencing of *HBA2, HBA1* genes revealed the presence of GGC (Gly) to CGC (Arg) substitution at codon 19 in *HBA2* (Fig. 2). Further alpha globin gene study (gap-PCR and MLPA) revealed this woman to be a carrier of the deletion $-\alpha^{3.7}$ (Fig. 3). The presence of α gene triplications was excluded.

Discussion

In this paper we report a case of simultaneous detection of Hb Handsworth and a $-\alpha^{3.7}$-thalassemia deletion mutation in an adult woman of Caucasisn origin. To the best of our knowledge this is the first report of such case in Poland. Thalassemia alpha, as well as thalassemia beta belong to a group of genetic disorders of hemoglobin synthesis both of which are rare in Northern Europe. Until quite recently these diseases were considered non-existent healthcare problems in Poland.

For many years the diagnosis of β-thalassemia both in our Institute laboratory and in other countries, was mainly based on the level of HbA_2 [14, 15, 16] and α-thalassemia remained undiagnosed. Following the implememntation of molecular techniques (gap-PCR, MLPA, sequencing of genes) we recognized several cases of α-thalassemia with various molecular backgrounds [5, 6].

The woman described in this paper has microcytosis and hipochromia with no evidence of iron deficiency. She had been given oral iron suplementation several times. Family history of hereditary anemia is unavailable.

Biochemical analysis for quantization of HbA_2 and HbF was normal but band migration with HbS position on alkaline electrophoresis and additional band on acid electrophoresis suggest that the patient has hemoglobinopathy. It is noteworthy that the presence of band migration at the HbS position on alkaline agarose gel can lead to misdiagnosis in the diagnostic schedules for thalassemia/hemoglobinopathy. We performed the sequencing of HBB gene and no mutations in this gene were detected. We also performed sequencing of *HBA2* and *HBA1* genes which revealed homozygous point mutation that results in the appearance of Hb Handsworth in *HBA2* gene. We then found that the

Table I. Hematological and biochemical data and patient's genotype

Parameters	Patient
RBC x 10^6/ul	5.04
Hb (g/dl)	12.5
MCV (fl)	72.6
MCH (pg)	24.8
MCHC (g/dl)	34.2
RDW %	14.3
Ferritin ng/ml	15.11
Iron ug/dl	65
HbA^2 (%)	2.2
HbF (%)	1.0
α-globin genotype	$-\alpha^{3.7}/\alpha\alpha^{Hw}$
ß-globin genotype	β/β

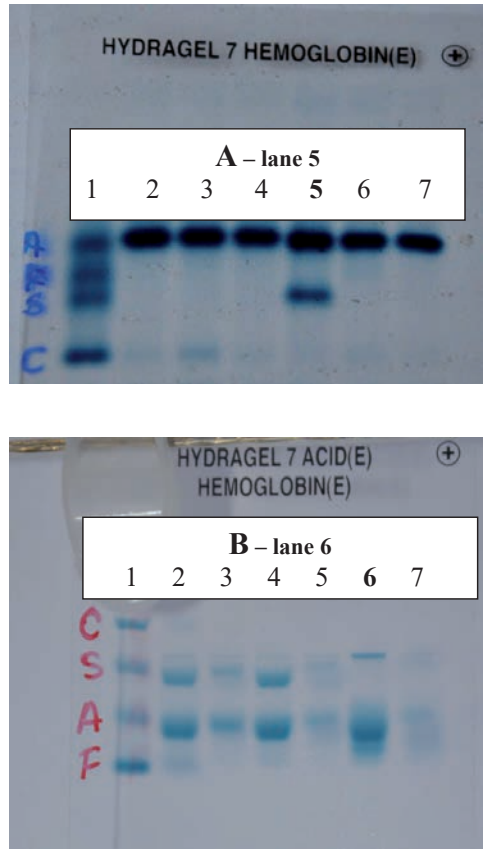

Fig. 1. Agarose gel electrophoresis at alkaline (A – lane 5) and acid pH (B – lane 6) showing bands in the A and S position (A – lane 5) and bands in A position and bands in the neighborhood of the band A position (B – lane 6)

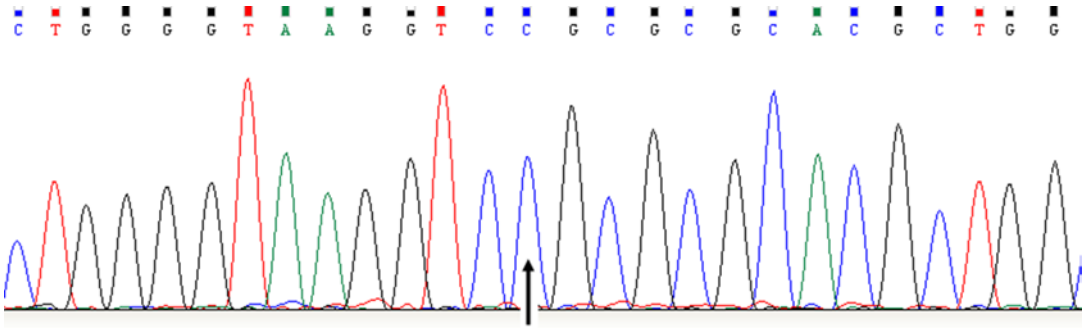

Fig. 2. DNA sequence analysis of the patient showing a GGC > CGC mutation at codon 19 of the* α2-globin *gene which results in the amino acid substitution (p.Gly19Arg).The arrow indicates the place of mutation

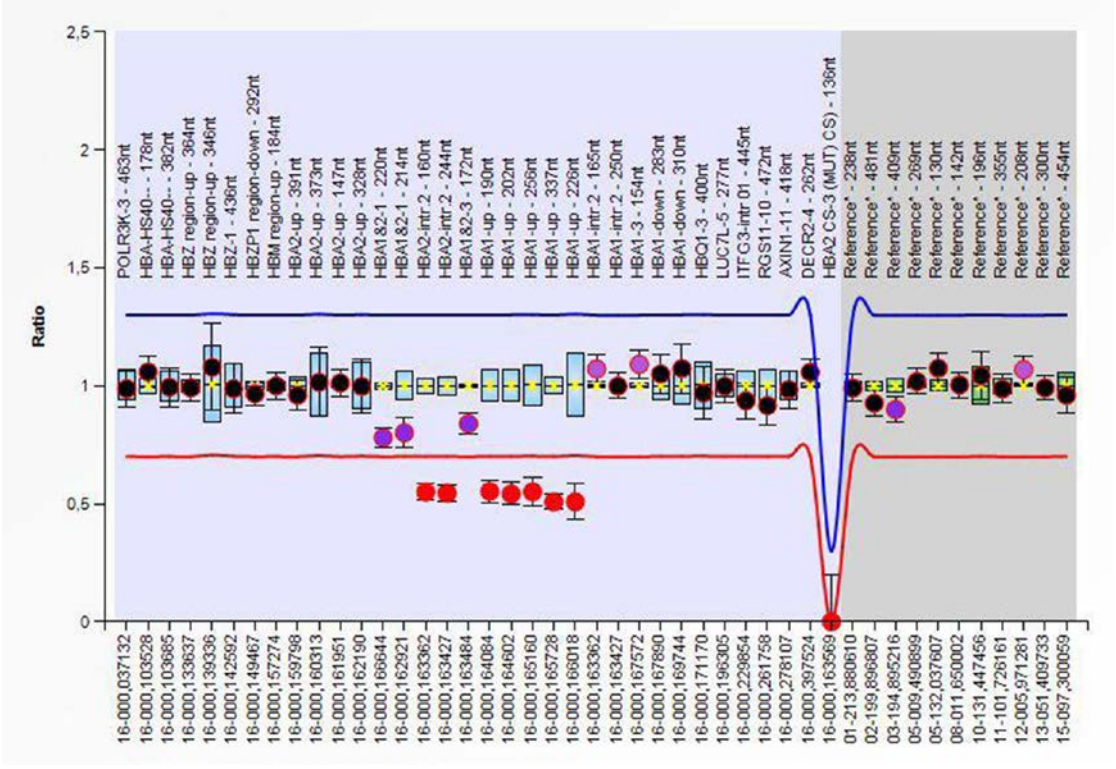

Fig 3. MLPA analysis of the patient's α-globin gene cluster allowed to detect the heterozygous deletion -α$^{3.7}$

patient carried a single gene -α$^{3.7}$ deletion. One must note that the primers specific for normal α-2 globin gene cannot amplify the fusion α gene on the chromosome with the -α$^{3.7}$ deletion. We must conclude that mutation: HBA2: c.55G>C is in *trans* to the -α$^{3.7}$ deletion.

Hb Handsworth like some α variants seems to be clinically asymptomatic (normal MCV and MCH) [3, 17, 18], but our case carries -α$^{3.7}$ deletion allele as well. Our double heterozygote however presented only mild anemia, which is typical for silent α- thalassemia phenotype.

It is noteworthy that Hb Handsworth diagnosis may be problematic as this hemoglobin "co-migrates" with HbS on agarose alkaline electrophoresis and creates very mild bands in acid electrophoresis. Routine use of molecular biology techniques such as gap-PCR, gene sequencing, and MLPA allowed for better defining of thalassemic genotypes (compound heterozygosity for abnormal hemoglobin and thalassemia mutations) and for correction of differential diagnosis of microcytic anemia.

We presented the molecular analysis of thalassemias taking into account the process of spreading multi-ethnicity in Northern Europe which results in growing consciousness of the problem assiociated with the common hemoglobinopathies such as HbS [19]. We must emphasize however that our particular case was detected in a Caucasian woman, which may suggest that alfa thalassemia and hemoglobinopathies are also present in the Polish population but were undetected prior to implementation of molecular biology methods. Confirmation of thalassemia diagnosis is important because

similar findings in blood morphology (microcytosis) are typical for iron deficiency; patients are therefore often exposed to unnecessary iron supplementation.

Authors' contributions/ Wkład autorów

The order of the authors reflects their participation in preparation of the manuscript.

Conflict of interest/ Konflikt interesu

There are no conflicts of interests.

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Ethics/Etyka

The work descibed in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to biomedical journals.

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