Beta Thalassemia IVS-I-5 (G→C) heterozygosity masked by the presence of HbJ-Meerut in a Dutch-Indian patient

We describe the genotype / phenotype correlation in a 35 years old anemic female referred to our laboratory because a fast eluting minor fraction on HPLC, mild hemolysis and hematological parameters suggesting a Thalassemia trait, eventually in combination with iron depletion. Direct sequencing of the alpha globin genes revealed heterozygosity for HbJ-Meerut, a Glu→Ala substitution at residue 120 not justifying the hematological parameters. No other point mutations were found on the α genes and Gap-PCR excluded the 6 common deletion defects. Direct sequencing of the ?-globin genes revealed the IVS-I-5 (G→C) transversion in absence of the elevated HbA^2 levels usually measured in carriers of this β-Thalassemia mutation. The HbA_2 tetramer in the presence of HbJ-Meerut divides in two parts. One $\alpha^N 2/\delta 2$ migrating on the right spot on HPLC. The other $\alpha^J 2/\delta 2$ migrating under the HbA fraction. Classic alkaline electrophoresis and the modern capillary electrophoresis CE showed these two tetramers and the reduction of the elevated HbA_2 level of the β-Thalassemia trait by at least 20% due to HbA_2 Meerut.

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Although some silent **β**-Thalassemia traits do not present with elevated HbA_2 fractions, the estimation of slightly to clearly elevated HbA_2 (3.5-8%) is the classic parameter associated with **β**-Thalassemia trait.

HbA_2 consists of two α and two δ polypeptide chains, hence abnormalities of the α-globin chains do influence the formation of HbA (α2/δ2), HbF (α2/δ2) and HbA_2 (α2/δ2) tetramers.

Stable abnormal hemoglobins induced by mutations on the α globin genes (HbX) will form an equivalent abnormal HbA_{2X}, usually expressed at the same ratio observed for the major HbA/HbX. Thus an average HbX resulting from an α1 gene defect, expressing at 15-20% of the HbA counterpart, will express at the same level an anomalous HbA_{2X}. This means that if the level of HbA_2 is 3% somewhere on the separation diagram a fraction of ± 0.5% corresponding to the HbA_{2X} should be visible, assuming that the sensitivity of the apparatus is sufficient and the location of the HbA_{2X} fractions is not overlapped by another (major) Hb fraction.

This observation is useful for the first judgement of an abnormal band or peak when, separating abnormal hemoglobins, one must decide which way to look for a DNA defect, on the α genes or on the β genes. In case of individuals with an elevated HbA_2 fraction because of β-Thalassemia heterozygosity the diagnostic HbA_2 level, usually higher than 4%, may become masked by the migration of part of the HbA_2 due to the presence of an ?-globin gene abnormality.

We report such a case in which the presence of a non-pathological HbJ-Meerut masks the presence of a risk bearing β-Thalassemia heterozygosity by reducing the HbA_2 level.

Patient

A 36 years old Dutch woman of Asian Indian origin, living in the city of Hengelo (Overijssel) was referred to our laboratory because of a fast eluting minor fraction on HPLC estimated at ±12%, mild hemolysis and hematological parameters suggesting a Thalassemia trait. The HbA_2 level was normal (3.2%) as well as ferritin, vit. B12 and folic acid.

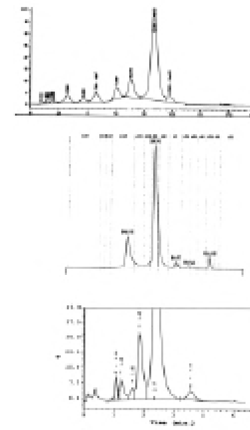

Figure 1. on top separation on the Agilent HPLC showing normal HbA2 and HbJ-Meerut as a faster fraction preceding HbA. In the middle CE separation showing the faste HbJ-Meerut some HbF, the extra HbA2 derived from the α globin Meerut and the normal non elevated HbA2. At the bottom HPLC on the Varian II showing some HbF, the fast HbJ-Meerut and a single but slightly elevated HbA2.

Figure 2. Part of the DNA sequencing of the α1 gene showing the GCG ? GAG transversion at cd 120 of the α1 gene, inducing heterozygosity for HbJ-Meerut.

Methods

The hematological indices were obtained on an automatic blood counter. Biochemical analysis included the classic separation and estimation of the Hb fractions. Hb separation was done in the local hospital laboratory using a in home assembled multi-components apparatus (Agilent 1100, Agilent Technologies, Palo Alto, CA, USA) provided with a PolyLC incorporation polyCatA Cation Exchange column (mobile phase Bis-Tris buffer with pH en NaCl gradient). Hb Separation at the reference lab was done on the *Variant-II* (Bio-Rad Laboratories, Hercules, CA, USA) using the β-thalassemia short program and on the *Capillarys 2* capillary electrophoresis apparatus (Sebia Lisses, France). In addition alkaline starch gel electrophoresis was performed. Genomic DNA was isolated at the local hospital laboratory and at the reference laboratory in Leiden by salt-extraction and modified gap-PCR

technology was used to screen for the six common α-thalassemia deletions (-α 3.7, -α 4.2, --MED-I, -α 20.5, --SEA, --FIL) and triplications (1, 2). Point mutation analysis of the β-globin gene was done on a GeneAmp9700 (Applied Biosystems, foster City, CA, USA) using the QIAGEN® Multiplex PCR kit (cat.no.206143). DNA sequencing was done on an ABI PRISMTM 3730 Genetic Analyzer (Applied Biosystems, foster City, CA, USA) using ABI PRISM® Big Dye Terminators v2.0 Cycle Sequencing Kit according to the instructions of the manufacturer as previously reported.

Results

Propositus presented at a first routine examination with abnormal hematological parameters (Hb 9 g/dl; Ht 0.29 l/l; MCV 60 fl; MCH 18.8 pg; RBC 4.78×10^{12}/l) in absence of iron depletion (Ferritin 52 μg/l). During a second examination 14 months later ferritin was 71 μg/l and ZPP 93 μmol/mol heme, indicating a normal iron level. However, the hematological parameters remained abnormal (Hb 10.2 g/dl; Ht 0.35 l/l; MCV 67 fl; MCH 19.8 pg; RBC 5.11×10^{12}/l). The Hb separation pattern was anomalous on electrophoresis and on the HPLC Agilent 1100 series, the HPLC Variant II and the Sebia CE devices. On all three apparatuses an anomalous fast moving peak (often considered as an aging artifact of the sample) was visible and was estimated at respectively 12%, 19.7% and 20.6%. The HbA_2 fraction was estimated at 3.2%, 3.9% and 3.4% on the three apparatuses, respectively. According to our normal range (2.5-3.4%) the HbA2 level was slightly elevated eventually indicating β-thalassemia heterozygosity only on the Variant II HPLC (Figure 1).

Gap-PCR failed to reveal any of the six common deletion defects associated with α-thalassemia (data not shown). Direct sequencing of the α globin genes revealed as the only abnormality a GCG→GAG transversion at cd 120 of the α1 gene, inducing heterozygosity for HbJ-Meerut, a non pathological Glu→Ala substitution, not justifying the hematological parameters in the propositus (Figure 2).

Direct sequencing of the β globin genes revealed heterozygosity for the common IVS-I-5 (G→C) transversion (data not shown) a severe β^+- Thalassemia defect, normally associated wit an elevated HbA_2 expression of 5% or more.

Discussion

HbJ-Meerut, also reported as HbJ-Birmingham is a stable, not pathologically relevant Hb variant, reported in families from Japan, India and Turkey. The variant has been described on both the α2 and the α1 gene by several authors (3, 4, 5, 6, 7). The expression reported was usually around 20% and 19.7%. This would mean an underestimation of about 40% on the HPLC Agilent 1100 and a correct estimation on both the Variant II HPLC and the CE apparatus. All systems were however able to identify this variant as an anomalous J fraction to be characterized at the molecular level. None of the three systems was able to identify the fraction directly, however other sample of Hb Meerut examined in our laboratory on the Variant I eluted approximately in the same window.

The Sebia CE separated most fractions showing also the $\alpha^J2/\delta2$ (0.7%) migrating between the HbA_2 (3.4%) and the HbF (3.1%). However the second HbF ($\alpha J2/\gamma2$) was overlapped by the HbA on CE. Assuming that this separation is the most accurate under this particular circumstances the HbA_2 should be 3.4 (A^2) + 0.7 (A_{2X}) = 4.1% indicating a carrier for β-Thalassemia. Using the HbJ-Meerut ratio and adding 20% to the 3.9% measured by the Variant II the HbA_2 would be 4.7%. This value is the nearest to the 5% reported in literature for the IVS-I-5 (G→C) mutation.

This shows that the carrier state of β-Thalassemia in this patient is still associated with the expected elevated HbA_2 level but also that this diagnostic parameter becomes useless due to the interference of HbJ Meerut. In such cases carrier diagnostics for high HbA_2 β-Thalassemia is bound to fail if the total hematological picture is not considered and if molecular analysis is not performed.

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