CASE REPORT

Rare Cases of Haemoglobin Variant Presented with Isolated Erythrocytosis

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ABSTRACT

Haemoglobinopathies presenting with erythrocytosis is relatively rare. The clinicians might mistakenly diagnose such patients with other causes of erythrocytosis such as myeloproliferative neoplasm, etc instead of haemoglobinopathies. Here, we described two cases of haemoglobin variant, namely Haemoglobin Johnstown (Hb Johnstown) and Haemoglobin Bethesda (Hb Besthesda) that were detected following various futile investigations for persistent erythrocytosis. For both cases, the two main screening methods used were capillary electrophoresis (CE) and high performance liquid chromatography (HPLC). Approximately 30% of the high affinity haemoglobin (Hb) are not detected by electrophoresis method, however, in some cases, a variant Hb peak can be seen in chromatography method. Thus, a high index of suspicion of such diagnosis is utmost important as to not to miss the definitive diagnostic test by DNA analysis.

Keywords: Erythrocytosis, Haemoglobin Johnstown, Haemoglobin Bethesda

INTRODUCTION

Haemoglobinopathies presenting with erythrocytosis is rare. Up to date, more than 100 haemoglobin variants with high oxygen affinity have been reported. Some of such haemoglobinopathies are Hb Malmo, Hb San-Diego, Hb Tak, Hb Johnstown, Hb Bethesda, etc. Most of the patients with these hemoglobinopathies are heterozygotes, except for Hb Tarrant which have been found in the homozygotic state (1).

Here, we report two rare cases of haemoglobin variant, namely Hb Bethesda and Hb Johnstown that were only detected after several unremarkable investigations performed for persistent erythrocytosis.

Hb Bethesda is a very rare haemoglobin variant. It is a beta globin variant where histidine is substituted for tyrosine at position 145 (β145 histidine). This substitution causes the haemoglobin structure to be more stable in its relaxed configuration, hence decreasing the release of oxygen. This ultimately lowers the P50 in oxygen dissociation curve, shifting the curve to the left. Hb Johnstown was first described in 1990 by Jones et al (1). This haemoglobinopathy is characterised by the presence of GTG→ATG mutation in codon 109 of the beta globin gene at exon 3 [B109(G11)Val→Leu]. The substitution of leucine for valine at position 109 produces a larger chain that alters the αβ structure of the haemoglobin molecule in the deoxy form, thus shifting the equilibrium towards high affinity oxy-haemoglobin.

CASE REPORT

The first case was a 49-year old diabetic lady who was incidentally noted to have persistent erythrocytosis. She was otherwise asymptomatic and was previously healthy. Further history revealed that her younger sister was also investigated for erythrocytosis, however the diagnosis was uncertain. Clinical examination showed no hepatosplenomegaly. Her Hb was 18.3g/dL, RBC 5.97x1012/L, haematocrit 0.552L/L, MCV 92.4fL and MCH 30.7pg. Her peripheral blood smear showed erythrocytosis with normal red cell and other blood cells morphology. In view of persistent erythrocytosis, a bone marrow aspirate (BMA) and trephine biopsy procedures were performed. The bone marrow aspirate showed normocellular (50% cellularity) marrow fragment for age, mild erythroid hyperplasia (myeloid : erythroid of 1:1) and normal blood cells morphology. Trehpine biopsy and cytogenetic analysis were unremarkable.
JAK 2 V617F mutation analysis showed a wild type and serum erythropoietin (EPO) level was normal. Hb analysis by HPLC showed a variant Hb peak (Figure 1). Hb electrophoresis at pH 8.5 showed that HbA band was present and no other abnormal bands were detected. DNA analysis of beta globin gene demonstrated the substitution of histidine for tyrosine at position 145 ($\beta$145 histidine): Hb Bethasda.

The second case was a 67-year old lady who also incidentally noted to be polycythaemic upon admission for dengue fever. Family history of similar problem was not documented. Her Hb was 18.9g/dL, RBC 9.32x10¹²/L, haematocrit 0.599L/L, MCV 64.2fL and MCH 20.3pg. This patient’s peripheral blood smear showed erythrocytosis and hypochromic microcytic red cells with normal other blood cells morphology. Bone marrow aspirate and trephine examination showed a hypercellular marrow fragment with erythroid hyperplasia (myeloid : erythroid ratio is 1:4) and normal morphology of all blood cells. Perls’ stain showed increase in iron stores with no ring sideroblast. JAK 2 V617F mutation analysis and calreticulin gene was not detected. Serum EPO level was normal, 34mIU/mL. Cytophenetic studies were normal. Hb analysis by (CE) and HPLC methods showed a raised HbA2 of around 5% (Figure.2a and Figure.2b respectively) which was compatible with beta thalassemia trait however, this did not correspond with the polycythaemic indices. DNA analysis of beta globin gene using Sanger sequencing using Big Dye cycle sequencing kit and analysed on the ABI 3730XL DNA Analyzer confirmed a compound heterozygous of codon 41/42 (-TTCT) Beta0 thalassemia and Codon 109 (GTG>TTG) Hb Johnstown.

DISCUSSION

Erythrocytosis occurs when there is an increase in red cell mass. Primary erythrocytosis occurs in the presence of an intrinsic defect in bone marrow causing an increase in red cell production. On the other hand, EPO induced red cell production results in secondary erythrocytosis. Most individuals with high affinity Hb are generally healthy and they are either diagnosed incidentally or after being noticed to be plethoric. Serum EPO levels are usually low in patients with primary erythrocytosis while, those with secondary erythrocytosis have normal or increased serum EPO levels. Study done by Galeas et al in 2015, demonstrated that patients with secondary erythrocytosis had a shorter time to death from time of diagnosis compared to those with primary erythrocytosis (4). Besides that, serum albumin levels were also lower in patients with secondary erythrocytosis compared to patients with primary polycythaemia vera (4).

Patients with high oxygen affinity Hb have mutation in their amino acid components, which are essential for the normal Hb functions. Such mutations causes alteration in the affinity of Hb to oxygen. This lowers the release of oxygen to tissue, causing tissue hypoxia, thus stimulating EPO production from the kidney leading to an increase in erythropoiesis. The high affinity haemoglobinopathy has been reported to have thrombotic complications among some patients (2).

Several Hb Bethesda and Hb Johnstown cases have been reported where almost all of those cases presented with incidental secondary polycythaemia due to inefficient oxygen dissociation from an abnormal haemoglobin (3). Such haemoglobinopathies are usually associated with significant family history. However, in the cases reported here, no family study were documented.

Hb Bethesda usually presents with cutaneous flush due to polycythaemia. However, this feature was not seen in our case. Cardiac and cerebrovascular complications have not been reported. (3) Patient’s serum erythropoietin level was similar to previously reported cases of Hb Bethesda (3). A case reported in Japan by Kawashima...
et al; 1994 showed an abnormal variant peak emerging after HbA and HbA2 as compared to small degree of shoulder at HbA2 peak observed in this case. Most carriers of Hb variants causing erythrocytosis are heterozygous, but some cases such as Hb Johnstown has been associated with B-thalassaemia, as compound heterozygous (5). This was also observed in our case, where Hb Johnstown was associated with Beta0 thalassemia. Ropero et al reported that erythrocytosis is more severe when a variant haemoglobinopathy is associated with B-thalassaemia (1). As such, the RBC count and HbA2 value are raised of 9.32×10¹²/L and 5.2% respectively due to the co-inheritance of disease as in current case. Most cases of Hb Johnstown/β0 thalassaemia need confirmation by Beta gene sequencing.

CE and HPLC sometime are used for further investigation of patient presenting with secondary erythrocytosis. Many of these high oxygen affinity haemoglobins are electrophoretically silent, however, some are well separated by HPLC technique. In such cases where the patients have secondary erythrocytosis but CE or HPLC does not show any significant results, definitive diagnostic test by DNA analysis of alpha and beta globin genes must be considered.

Patients who have symptoms associated with erythrocytosis should be managed by multidisciplinary medical team. According to Yudin J et al, the treatment of high oxygen affinity Hb is uncertain and has to be based on the clinical presentation (2). Venesection to reduce the haematocrit levels and anti-platelet therapy should be considered to prevent thrombotic episode (2).

CONCLUSION

Presentation of erythrocytosis can be a diagnostic challenge to physicians, leading to several pointless investigations. An isolated erythrocytosis with the absence of other secondary causes of erythrocytosis should prompt the possibility of a high oxygen affinity haemoglobinopathy. Thus, a high index of suspicion among clinicians would allow proper diagnostic strategies to be done. Molecular analysis is mandatory in such cases when screening methods by CE and HPLC are inconclusive. It is highly recommended that all adult cases with polycythaemia should be checked for haemoglobinopathy to rule out secondary erythrocytosis.

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REFERENCES


