



Original Article

Markedly elevated fetal hemoglobin in myeloid neoplasms: A potential diagnostic pitfall during hemoglobinopathy work-up

Tushar Sehgal¹, Sweta Rajpal¹, Narender Kumar¹, Shano Naseem¹, Sanjeev Chhabra¹, Amita Trehan², Pankaj Malhotra³, Reena Das¹, Prashant Sharma¹

¹Department of Hematology, ²Department of Pediatrics, Pediatric Hematology Oncology Unit, ³Department of Clinical Hematology and Medical Oncology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

*Corresponding author:

Prashant Sharma,
Department of Hematology,
Postgraduate Institute of
Medical Education and
Research, Chandigarh, India.

sharma.prashant@pgimer.edu.in

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ABSTRACT

Objectives: Screening tests, including high-performance liquid chromatography (HPLC) for the diagnosis of thalassemia syndromes, are based primarily on the detection of markedly elevated levels of fetal hemoglobin (HbF). However, several uncommon acquired conditions can also occasionally raise HbF levels to those similar to hemoglobinopathies. Very high HbF levels in myeloid neoplasms may reflect an extreme form of stress erythropoiesis, i.e., a reversion to a fetal phenotype characterized by red cell macrocytosis, reduced HbA₂, increased *i* antigen expression, an excess of $\epsilon\gamma$ over $\alpha\gamma$ globin chains, and low carbonic anhydrase levels with a fetal-type isozyme pattern. This study aims to explore the diagnostic challenges of elevated HbF on HPLC and highlight the value of combining morphological assessment and parental studies to improve the accuracy of thalassemia diagnosis.

Materials and Methods: Cases with elevated HbF on HPLC were identified from laboratory archives, and clinical data were retrieved from the case files. Inherited hemoglobinopathies were excluded by the absence of prior suggestive history and confirmed by parental HPLC studies.

Results: We report five male patients with unusually high acquired HbF levels associated with acute myeloid leukemia (AML) with myelodysplasia-related changes (a 24-year-old with 46.1% HbF), acute erythroleukemia (a 3-year-old with 38.9% HbF), and three juvenile myelomonocytic leukemia (JMML) patients (3-year-old with 44.2% HbF, 3-year-old with 58.2% HbF, and 5-year-old with 38% HbF).

Conclusions: Recognizing dysplasia and increased blasts in blood and marrow during HPLC interpretation helped prevent misdiagnosis of thalassemia syndrome and unnecessary work-ups. Blood film review and parental HPLCs should be essential steps in all suspected thalassemia cases.

Keywords: Erythropoiesis, Fetal hemoglobin, Hemoglobinopathy, High-performance liquid chromatography, Myeloid neoplasms

INTRODUCTION

Fetal hemoglobin (HbF) is the dominant form of hemoglobin (Hb) present in the fetus during the gestational period.^[1] It is produced by precursor cells of erythroid lineage starting from 10-12 weeks of pregnancy through the first 6-12 months of postnatal life. HbF contains 2 alpha

and 2 gamma subunits, while the major form of adult Hb, hemoglobin A (HbA), contains 2 alpha and 2 beta subunits.^[1-3] HbF constitutes 60-80% of total Hb in the full-term newborn. By approximately 6-12 months of age, it is almost completely replaced by HbA. HbF levels in adults are typically <1.0%.^[2]

Increased HbF levels may be encountered in adults, mainly in association with β -globin synthesis abnormalities.^[4] These inherited conditions include hereditary persistence of HbF, the β -thalassemia syndromes, sickle cell anemia, and hereditary spherocytosis, as well as various heritable bone marrow failure syndromes. Several acquired conditions are also known to increase HbF. These include stress erythropoiesis, pregnancy, aplastic anemia, pernicious anemia, diabetes mellitus, thyrotoxicosis, and infections such as kala-azar, hepatic tumors, and various drugs. In nearly all of these, the rise in HbF levels is variable and usually small (typically <5%).^[4,5]

Myeloid neoplasms, especially acute myeloid leukemia (AML), juvenile myelomonocytic leukemia (JMML), the myelodysplastic neoplasms (MDS), and polycythemia vera are known to have inconsistent increases in HbF levels.^[6] It reflects stress erythropoiesis, ineffective erythropoiesis, or epigenetic dysregulation of globin gene expression.^[6] Levels >10% also serve as poor prognostic indicators in such cases.^[6,7] However, very high HbF levels are relatively uncommon in most published series. We report five patients, two with AML and three with JMML, where unusually high and diagnostically potentially confusing HbF levels were encountered.

Objectives

This study aims to explore the diagnostic challenges of elevated HbF on HPLC and highlight the value of combining morphological assessment and parental studies to improve the accuracy of thalassemia diagnosis.

MATERIALS AND METHODS

This was a retrospective observational study conducted in the Hemolytic and Nutritional Anemia Laboratory of Post Graduate Institute of Medical Education and Research, Chandigarh, India. The laboratory archives were systematically searched for cases demonstrating elevated levels of HbF, as estimated by high-performance liquid chromatography (HPLC).

The study period included samples processed between January 2013 and December 2015. All cases in which HPLC analysis showed elevated HbF beyond the laboratory reference range were retrieved. The laboratory reference ranges for HbF are age dependent: 60-80% in newborns (physiological), <5% by 6 months of age, <2% in children

aged >1 year, with a gradual decline to the adult reference range of <1.0%.^[2] Relevant demographic details (age, sex), hematological parameters, and additional laboratory data were noted. Clinical information, including provisional and final diagnoses and clinical presentations, was accessed through the clinical notes. Data were anonymized before analysis to maintain confidentiality. The final dataset was compiled in Microsoft Excel and analyzed descriptively to determine the spectrum of disorders associated with elevated HbF.

RESULTS (CASE DETAILS)

Case 1

A 24-year-old male presented with fever and generalized petechial hemorrhages for 15 days. On examination, he had no organomegaly or significant lymphadenopathy. Hemogram revealed Hb 97 g/L, total leukocyte count (TLC) $22.3 \times 10^9/L$, and platelet count $19 \times 10^9/L$. A blood film showed leukoerythroblastosis with 32% blasts, 1% myelocytes, 1% metamyelocytes, and 12 nucleated red cells per 100 leukocytes. Bone marrow aspirate showed 45% erythroid cells and 29% blasts that were cytochemically positive for myeloperoxidase stain along with 45% erythroid cells. Dysgranulopoiesis and dysmegakaryopoiesis were present in 70% and 55% of the respective lineage precursors. On flow cytometry, the blasts expressed CD13, CD33, CD117, cytoplasmic myeloperoxidase, and, human leukocyte antigen – DR, and polymerase chain reaction, respectively. They were negative for monocytic and B/T-lymphoid markers. Multiplex reverse-transcriptase PCR was negative for t(15;17), t(8;21), and inv(16). A final diagnosis of AML with maturation (French–American–British, FAB classification subtype AML-M2) and multi-lineage dysplasia was rendered.

Hb HPLC had been ordered at admission, before the blood film results were obtained. It showed markedly elevated HbF (46.1%), reduced HbA2 (1.4%), and 50.7% HbA0 (adult Hb) (Bio-Rad Variant II system using the β -Thal Short program; Bio-Rad Laboratories, Hercules, CA, USA). The possibility of an incidentally detected mild thalassemia intermedia was considered by the diagnostic service. However, perusal of the blood and marrow reports and with the patient denying any prior history of anemia, jaundice, or transfusions, this was deemed unlikely. Hb HPLCs of both his parents were normal, excluding an inherited thalassemia syndrome.

Case 2

A 3-year-old previously healthy boy, a resident of Himachal Pradesh, India, presented with listlessness and low-grade fever for 2 months. There was no history of jaundice or exposure to tuberculosis, and he was appropriately immunized for his age. Examination revealed hepatosplenomegaly, each 3 cm below

the respective costal margins. No stigmata of Fanconi anemia or neurofibromatosis were detected. Hemogram showed pancytopenia; Hb 87 g/L, corrected reticulocyte count 4%, platelet count $22 \times 10^9/L$, and TLC $33 \times 10^9/L$. Peripheral blood film showed leukoerythroblastosis with 4% atypical blastoid cells, and 110 nucleated red cells per 100 leukocytes. Red cells showed mild anisocytosis and were normocytic normochromic with occasional macrocytes and a few polychromatophils. Himachal Pradesh has kala-azar endemic zones; hence, RK39 tests and Napier aldehyde test were done. The former was negative while the latter was non-specifically positive. Since northern India carries a high burden of β -thalassemia carriers, Hb HPLC was also performed. It showed HbF 38.9 % and HbA2 1.2%. Both parents' HPLCs were normal, and this, together with the anomalous red cell morphology, made a thalassemia syndrome unlikely.

Bone marrow aspiration was done in view of the circulating blasts. It revealed 24% myeloperoxidase-negative blasts along with 75% megaloblastic erythroid precursors with significant dyserythropoiesis. Granulocytic cells and megakaryocytes were reduced. Flow cytometry revealed myeloid marker expression by the blasts (CD13, CD33, and CD117). Reverse-transcriptase PCRs were negative for t(9;22), t(15;17), t(8;21), and inv(16). The case was signed out as acute erythroleukemia, erythroid-myeloid (AML M6a).

Case 3

A 3-year-old boy presented with fever and lethargy for 1 month. Physical examination revealed hepatosplenomegaly. Hemogram showed Hb 89 g/L, platelet count $70 \times 10^9/L$, TLC $60.3 \times 10^9/L$ with an absolute monocyte count of $9.0 \times 10^9/L$. Peripheral blood showed normocytic normochromic anemia, 4% circulating blasts (including promonocytes), and 15% monocytes along with a mild neutrophilic maturational shift-to-the-left. Nucleated red cells were not seen. Bone marrow was hypercellular and showed 7% blasts along with an increase in monocytic and granulocytic elements. The erythroid and megakaryocytic lineage cells were relatively reduced. Hb HPLC showed 44.2% HbF. Parental Hb HPLCs were both normal. Reverse transcriptase PCR for BCR-ABL1 fusion was negative. A diagnosis of JMML was made.

Case 4

A 3-year-old boy presented with fever and lethargy for 1 month. Physical examination revealed hepatomegaly. Hemogram showed Hb 66 g/L, platelet count $37 \times 10^9/L$, TLC $24.3 \times 10^9/L$ with an absolute monocyte count $2.7 \times 10^9/L$. Peripheral blood showed normocytic normochromic anemia, 05% circulating blasts + promonocytes, and 11% monocytes along with a mild neutrophilic shift to the left.

Bone marrow was hypercellular and showed 08% blasts along with an increase in monocytic and granulocytic elements. The erythroid and megakaryocytic lineages were relatively reduced. Hb HPLC showed 38% HbF. Parental Hb HPLCs were both normal. A diagnosis of JMML was made.

Case 5

A 3-year-old boy presented with fever and lethargy for 1 month. Physical examination revealed hepatosplenomegaly. Hemogram showed Hb 83 g/L, platelet count $14 \times 10^9/L$, TLC $28 \times 10^9/L$ with an absolute monocyte count of $5.3 \times 10^9/L$. Peripheral blood showed normocytic normochromic anemia, 01% circulating blasts (including promonocytes), 19% monocytes, and 02% myelocytes. Bone marrow was hypercellular and showed 7% blasts along with an increase in megakaryocytic and granulocytic elements. The erythroid lineage was relatively reduced. Hb HPLC showed 58.2% HbF. Parental Hb HPLCs were both normal. A diagnosis of JMML was made.

Table 1 presents the summary of hematological findings in the cases with acquired causes of raised HbF.

DISCUSSION

Among the hematological malignancies, elevated HbF levels are described in AML, myeloproliferative neoplasms (MPN), MPN/MDS overlap syndromes, and Hodgkin and non-Hodgkin lymphomas.^[8-10] Very high HbF levels potentially simulating β -thalassemia syndromes on laboratory results have previously been reported by Sheridan *et al.*^[9] in JMML (mean \pm SE 35.0 ± 8.9 , range 4.9-70%) and infantile acute erythroleukemia (mean \pm SE 8.07 ± 4.6 , range 0.7-41.7%). HbF levels in our five cases were comparable with those in the upper half of their respective diagnostic categories in that study. Interestingly, although nearly 2/3 of their cases of AML, acute lymphoblastic leukemia, and chronic myeloid leukemia showed increased HbF, in none of the cases did the levels cross 25%. Case 1 with AML, therefore, appears anomalous till one considers that the patient also had multi-lineage dysplasia. The specific category of AML with myelodysplasia-related changes is known to have considerable overlaps with acute erythroleukemia, and our patient with 45% erythroid cells could have had a biologically similar disease.

The elevated HbF in JMML is best explained as an exaggerated form of stress erythropoiesis, with relentless progression, as the disease progresses, toward a form of nearly completely fetal erythropoiesis. This is evidenced by red cell macrocytosis, reduced HbA2, increased expression of the *i* antigen, an excess of $\text{C}\gamma$ than $\text{A}\gamma$ globin chains, and low carbonic anhydrase levels with a fetal type isozyme pattern.^[9-11] However, the diagnostic specificity of these and other findings has been challenged. There exist reports

Table 1: Summary of hematological findings in the cases with acquired causes of raised fetal hemoglobin.

Case no.	Age (years)	Organomegaly/Lymphadenopathy	Hb (g/L)/Platelet count ($\times 10^9/L$)/TLC ($\times 10^9/L$)	Peripheral blood and bone marrow findings	Molecular/Cytogenetic testing	HbF (%)
1	24	None	97/19/22.3	AML with myelodysplasia-related changes	<i>t</i> (15;17)- Neg <i>t</i> (8;21)- Neg <i>inv</i> (16)- Neg	46.1
2	3	Hepatosplenomegaly	87/22/33	Acute erythroleukemia, erythroid-myeloid	<i>t</i> (15;17)- Neg <i>t</i> (8;21)- Neg <i>inv</i> (16)- Neg <i>t</i> (9;22)- Neg	38.9
3	3	Hepatosplenomegaly	89/70/60.3	Peripheral blood showed monocytosis with leukoerythroblastic picture; Bone marrow is suggestive of JMML	<i>t</i> (9;22)- Neg	44.2
4	5	Hepatomegaly	66/37/24.3	Peripheral blood showed monocytosis with leukoerythroblastic picture; Bone marrow is suggestive of JMML	<i>t</i> (9;22)- Neg FISH for monosomy 7- Neg	38
5	3	Hepatosplenomegaly	83/14/28	Peripheral blood showed monocytosis with leukoerythroblastic picture; Bone marrow is suggestive of JMML	<i>t</i> (9;22)- Neg	58.2

of persistent EBV infection in two infants with findings consistent with JMML, including increased numbers of F and *i* cells and abnormal granulocyte-macrophage colony formation *in vitro*. Both showed complete and sustained recovery without treatment.^[11] Fluhr *et al.*^[12] reported that elevated HbF in JMML results from epigenetic dysregulation of KLF1, leading to impaired repression of γ -globin genes at the β -globin locus and persistence of HbF expression. They concluded that an increased HbF in JMML originates in the clonal leukemic erythropoiesis and is distinct from elevated levels of HbF observed under certain circumstances of stress erythropoiesis or in non-malignant hematopoietic disorders such as β -thalassemia or sickle cell disease.^[12] Clinical risk stratification in JMML incorporates age at diagnosis, platelet count, and age-adjusted HbF levels as key prognostic variables.^[13,14] In a large JMML cohort, age >2 years at diagnosis, platelet count $<33 \times 10^9/L$, and HbF levels $\geq 10\%$ were identified as the strongest predictors of poor survival.^[13] Building on these observations, Passmore *et al.*^[15] developed a prognostic scoring system in which HbF $\geq 10\%$ and platelet count $\leq 33 \times 10^9/L$ were assigned adverse prognostic significance.^[15]

Pagnier *et al.*^[10] reported an unusual case of leukemia with high HbF. The patient presented without any apparent hematologic disorders except for moderate anemia. There were no Hb abnormalities in the parents that led to a suspicion of a latent malignancy that, on follow-up, was confirmed to be myelomonocytic leukemia. The expression of red cell antigen *i* was increased, while those of I, A, and A₁ antigens decreased progressively. Two populations of erythrocytes, A-positive and A-negative, were distinguished. The unbalanced globin chain synthesis, increase HbF, and antigenic changes of the membrane were restricted to the A-negative population. The biologic data were not entirely

consistent with a reversion to fetal erythropoiesis. The question remains unanswered of a polychromosomal lesion of either quiescent F cells or adult stem cells.^[10]

The major reason we present these cases is to remind and to alert hematopathologists reporting Hb HPLCs and other Hb studies, like electrophoresis, that such high levels of HbF will occasionally be encountered in patients with myeloid malignancies. They illustrate the utility of knowing the complete clinical background, which, together with a blood film evaluation, can help avoid misdiagnosis, especially in pediatric patients with hepatosplenomegaly and anemia. The availability of the history, blood film, and reticulocyte count can also increase diagnostic yield by enabling pick-up of cases with normal HPLCs but showing anemias with typical red cell morphology (spherocytes, schistocytes, elliptocytes, stomatocytes, etc.) during HPLC sign-outs.

In confusing cases, parental Hb HPLCs can inexpensively and rapidly exclude homozygous or compound heterozygous hemoglobinopathies, proving invaluable in our study. Both JMML and acute erythroleukemia may be slower in evolution than acute leukemias, and other differential diagnoses are entertained till the diagnostic hematological profiles are recorded.

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CONCLUSIONS

Recognizing dysplasia and increased blasts in peripheral blood and bone marrow during HPLC interpretation is crucial to avoid misdiagnosis of thalassemia syndromes and

unnecessary investigations. Careful peripheral smear review, along with parental HPLC evaluation, should be considered essential steps in the diagnostic work-up of all suspected thalassemia cases.

Ethical approval: Institutional Review Board approval is not required as it is a retrospective study.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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