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Clinical Significance of Elevated Fetal Hemoglobin in Adult Patients Hospitalized in "University Hospital Center Mother Theresa, Tirana"

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Abstract

Background: Fetal Hemoglobin (HbF $\alpha 2\gamma 2$) synthesis declines during the third trimester and is gradually replaced to adult hemoglobin (HbA $\alpha 2\beta 2$) resulting in less than 1% HbF in normal adults. In several situations during medical practice, abnormally high values of HbF represent a challenge to the clinicians.

Aims: The aim of this study is to create a profile of acquired and inherited disorders that lead to elevated values of HbF in adult patients, based on their electrophoretic pattern.

Study Design: This is a cross-sectional study.

Methods: Whole blood K2-EDTA samples were analyzed by Alkaline Gel Electrophoresis, Sebia Hydrasis. HbF presence was confirmed by alkali denaturation test. Data from patients hospitalized in "University Hospital Centre Mother Theresa" from January 2015 to January 2018 were analyzed and adult patients with abnormal HbF values were selected as our subject.

Results and Discussion: 124 patients present elevated HbF in Hemoglobin Electrophoresis performed in our laboratory. 55.6% (69 patients) had HbF above 10% and 44.4% (55 patients) had HbF less than 10%. InHbF>10% category, 7 patients (10%) had Thalassemia Major, 47 patients (68%) had Drepanocytosis (68% with normal HbA2, 32% with HbA2>3.5%). 15 patients (22%) showed HbF values 10-20% with possible diagnosis Hereditary Persistence of Fetal Hemoglobin (HPFH), $(\delta\text{-}\beta^{-})$-Thalassemia Carriers or Sardinian $(\delta\text{-}\beta)$ Thalassemia Heterozygotes. In HbF<10% category, 29 patients (53%) have Thalassemia Minor, 2 patients (4%) present

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borderline HbA2, which diagnosis should be determined between carriers of β-Thalassemia silent mutations, α-gene triple locus or δ-β-Thalassemia Heterozygotes and 24 patients (43%) have HbA2<3.2%. Such values might be due to Iron-Deficiency Anemia, δ-globin anomaly coexistence, Aplastic Anemia, Acute/Chronic Myeloid Leukemia, Myelodysplasia, HbH-β^{+}Thalassemia Trait, etc.

Conclusion: HbF is an important diagnostic parameter in various hematological disorders that should be known by clinicians. Elevated HbF with HbA2<3.2% requires further investigation.

Key words: Fetal Hemoglobin, adults, diagnostic profile

INTRODUCTION

Hemoglobin Hb in normal adults includes 96-98% HbA with the molecular structure α2β2, up to 3.5% HbA2 α2δ2 and less than 1% HbF α2ɣ2. HbF in adults is confined to a subset of red blood cells RBC called F cells, which constitute about 3-7% of the erythrocytes, containing 20-25% of HbF (1, 2, 3). HbF is produced from the sixth week of gestation and during the rest of fetal life, replacing the embryonic hemoglobins Gower I, Gower II and Portland. HbF is heterogeneous, with two types of ɣ-chains having either glycine ($ɣ^G$chains) or alanine ($ɣ^A$ chains) at position 136. $ɣ^G$ and $ɣ^A$ globin chains are encoded by two distinct genes located in the β-globin cluster on chromosome 11. The $γ^G$:$γ^A$ ratio is around 70:30 at birth and usually 40:60 in the trace amount of HbF found in the adult (4). Shortly after the time of birth there is a switch from predominant expression of HbF to adult hemoglobin (HbA), which is mediated by a transcriptional switch in definitive erythroid progenitors from *γ*- to *β-globin* (1).

HbF has a high affinity for oxygen due to its inability to interact with 2,3-diphosphoglycerate. High oxygen affinity of HbF allows it to bind to oxygen more avidly than normal hemoglobin, leading to tissue hypoxia in adults, increased erythropoietin and an elevated mass of RBCs. Furthermore, studies in fetuses, children with cyanotic heart disease and adults ascending to high altitude suggest that HbF synthesis increases in hypoxia (5, 6, 7).

There are several situations during medical practice in which abnormally high values of HbF represent a real challenge to the clinicians. The aim of this study is to create a profile of acquired and inherited disorders that lead to elevated values of HbF in adult patients, based on their electrophoretic pattern.

MATERIAL AND METHODS

This is a cross-sectional study performed in Laboratory Department, Hospital Center 'Mother Theresa' Tirana. We retrospectively collected data from Hemoglobin Electrophoresis in a three years period, from January 2015 to January 2018. Our subjects were adult patients with abnormally elevated HbF values. SPSS was used for statistical analysis. We ran Descriptive Statistics and used Independent Samples t-test for comparison between groups. P-value<0.05 was considered statistically significant.

All samples were collected in K2-EDTA anticoagulant tubes. Electrophoresis on alkaline agarose gels (pH 8.5), SebiaHydrasys was used for the separation of hemoglobins and detection of hemoglobin variants. The assay is performed on the hemolysate from washed red blood cells. Different hemoglobins migrate differently depending on the molecule's charge. Fractions are visualized by staining with amidoblack. The resulting electrophoregrams were first evaluated visually for pattern abnormalities and then scanned densitometrically. Densitometry serves in the interpretation by providing relative concentrations of individual fractions (8).

We confirmed the presence of HbF in our samples by alkali denaturation as a qualitative test. The

resistance to alkaline denaturation seen in fetal cells in the first rapid reaction with NaOH is attributed to the presence of HbF (9).

RESULTS

124 patients present elevated HbF values in Hemoglobin Electrophoresis performed in our laboratory from January 2015 to January 2018. 54% were females and 46% were males. Mean value of HbF resulted 17.8%, with a minimum of 1.9%, a maximum of 98.7% and Standard Deviation 16.5.

We categorized our patients in two major groups according to their HbF value. 55.6% (69 patients) had HbF above 10% and 44.4% (55 patients) had HbF less than 10%. (Figure 1)

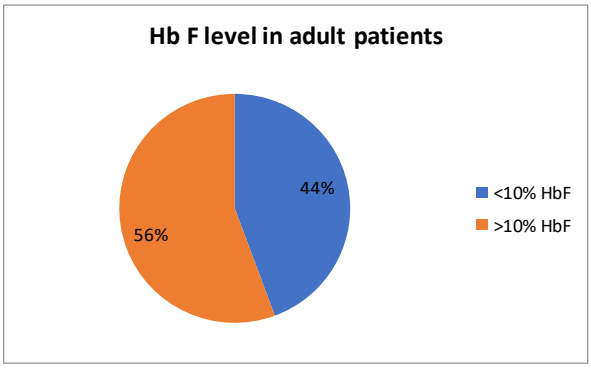

Figure 1. HbF level in adult patients

HbF>10%

In HbF>10% category, 7 patients (10%) had Thalassemia Major, 47 patients (68%) had Drepanocytosis, 15 patients (22%) showed HbF values 10-20%. (Figure 2)

Electrophoretic examination doesn't offer enough information to determine the diagnosis of patients with HbF values 10-20%. HPFH, (δ-β$^-$)-

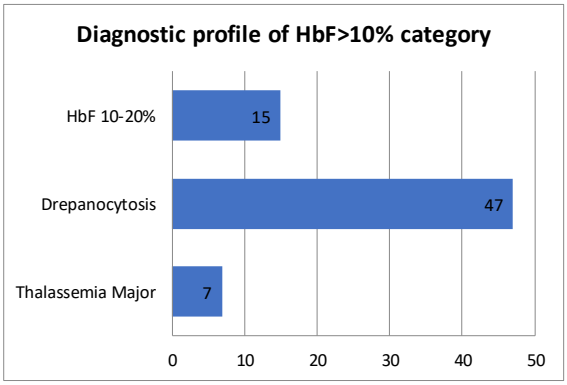

Figure 2. Diagnostic profile of patients with HbF>10%

Thalassemia Carriers and Sardinian (δ-β) Thalassemia Heterozygotes are possible diagnosis since their HbF level is within this range.

Patients suffering from Drepanocytosis were further categorized in two groups according to their HbA2 level. 68% show HbA2 within the normal range and 32% show HbA2>3.5%. Independent Samples t-test showed a significant difference (p=0.01) among HbF values in these two groups. (Table.1)

Table 1. Comparison of HbF values in patients with Drepanocytosis

HbA2 in Drepanocytosis	Number of patients	Mean HbF	Std. Deviation	p-value
HbA2</=3.5%	32	23.87	8	0.01
HbA2>3.5%	15	17.82	7.78	

HbF<10%

Differential diagnosis of patients with HbF values less than 10% is assessed through the evaluation of HbA2. In HbF<10% category 29 patients (53%) have HbA2>3.5%, 2 patients (4%) present

borderline HbA2 (3.2-3.5%) and 24 patients (43%) have HbA2<3.2%. (Figure 3)

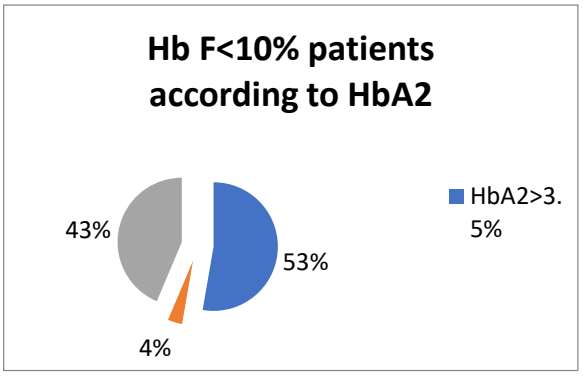

Figure 3. Classification of patients with HbF<10% according to HbA2 values

HbA2 values above 3.5% determine Thalassemia Minor, so 29 subjects from our study are carriers of this trait.

The diagnosis of patients in borderline HbA2 group should be determined between carriers of β-Thalassemia silent mutations, α-gene triple locus or δ-β- Thalassemia Heterozygotes.

Elevated HbF (<10%) combined with HbA2 less than 3.2% might be due to Iron-Deficiency Anemia, δ-globin anomaly coexistence, Aplastic Anemia, Acute/Chronic Myeloid Leukemia, Myelodysplasia, HbH-β^+Thalassemia Trait, etc.

DISCUSSION

Careful interpretation of the electrophoretic pattern is not enough to establish a specific diagnosis in many adult patients with elevated values of HbF. Complete Blood Count and peripheral blood films offer useful information and should be interpreted simultaneously. Our study is limited by the lack of clinical data and the impossibility to further confirm our findings by molecular biology. In the following paragraphs we explore the causes of elevated HbF in adults based on their electrophoretic pattern and discuss some of the confusing diagnostic entities with similar HbF values.

Elevated HbF values are characteristic of various acquired and inherited disorders. The level of HbF is increased in acquired states such as pregnancy, aplastic anemia, thyrotoxicosis, hepatoma, myeloproliferative disorders like Juvenile chronic myeloid leukemia, megaloblastic anemia, recovery from marrow hypoplasia, recovery from bone marrow transplantation, or hypoplastic myelodysplastic syndrome (10).

Most of the inherited conditions with increased HbF levels are associated with β-globin synthesis abnormalities (5, 10). Deletions affecting the β-globin gene cluster result in the syndromes of β-thalassemia, hereditary persistence of fetal hemoglobin (HPFH), δβ-thalassemia, and γδβ-thalassemia (11). HPFH is a benign asymptomatic condition in which HbF synthesis continues into adulthood. Beta-thalassemia is caused by the reduced (beta$^+$) or absent (beta^0) synthesis of the beta globin chains of the hemoglobin tetramer. The Beta-thalassemia carrier state results from heterozygosity for beta-thalassemia and is defined by characteristic hematologic features: microcytosis, hypochromia, HbA2>3.5 and variable amount of HbF. In beta^0-thalassemia, HbA is absent, HbF is 95-98% and HbA2 is 2-5%. In beta$^+$-thalassemia homozygotes with a residual variable beta globin synthesis or beta^0/beta$^+$

compound heterozygotes, the Hb pattern shows HbA 10-30%, HbF 70-90% and HbA2 2-5% (12). A confusing scenario is the diagnosis of Beta Thalassemia Trait with normal or borderline values of HbA2 and elevated HbF. Decreased HbA2 levels can be detected in iron depletion, possibly due to the preferential binding of β to α chains rather than δ chains or to an inhibition of low iron levels on δ globin synthesis (13, 14).

δβ-Thalassemia results from the deletion of both δ and β genes. Homozygotes for δβ-thalassemia have 100% HbF. The phenotype of heterozygotes resembles that of the β-thalassemia trait, but the HbA2 percentage is not increased and is often normal. HbF in such individuals is consistently elevated, varying from 5% to 20%. Peripheral blood film findings are similar to those for the β-thalassemia trait and the distribution of HbF is heterocellular, which is best observed via flow cytometry. It is necessary to distinguish it from hereditary persistence of fetal hemoglobin. The two groups of disorders are distinguished by the phenotype of heterozygous individuals. Heterozygotes of δβ-thalassemia mutations have 5% to 20% HbF, which is heterocellularly distributed in red cells, whereas heterozygotes of HPFH mutations have 17% to 30% HbF, with a pancellular distribution. In addition, homozygotes of HPFH are asymptomatic, whereas δβ-thalassemic homozygotes have thalassemia intermedia-like features (15, 16).

Drepanocytosis or Sickle-cell disease (SCD) is an allelic disorder, located on the chromosome 11, at 11p15, with an autosomal recessive pattern of inheritance. SCD occurs as a result of a mutation at a single nucleotide (A to T) of the β-globin gene, which results in glutamic acid being substituted by valine at position 7. The most important predictor of SCD severity is HbF level. Benefits of high HbF derive from its exclusion from the sickle hemoglobin polymer. Individuals with high levels of HbF experience milder forms of the disease with lower morbidity and improved survival. HbF level of 10% and above is believed to reduce the risk of major organ failure such as stroke, while much higher levels (20% and above) may be required to prevent recurrent clinical events such as painful crises and pulmonary disorder. Stimulation of HbF production is the target of SCD treatment with hydroxycarbamide (Hydroxyurea) (17, 18, 19).

The elevated value of HbA2 may produce diagnostic confusion within drepanocytosis and Hb S/β-thalassemia. Microcytosis is not a feature of SCD, while patients with HbS/β-thalassemia typically exhibit microcytosis. SCD and HbS/β^0-thalassemia patients do not have any HbA, unless the patient has been transfused or has undergone red cell exchange. HbS/α-thalassemia is considered when the percentage of HbS is lower than expected. Classical cases of sickle cell trait are 60% of HbA and approximately 35–40% of HbS. Cases of HbS/α-thalassemia will have lower values of HbS, typically below 30% with microcytosis. A similar picture will also be present in patients with sickle cell trait and iron deficiency (20). (Table. 2)

Table 2. Laboratory differentiation of sickle cell anemia, sickle cell anemia/α-thalassemia and HbS/β^o-thalassemia (21)

Diagnosis	Hemoglobin(g/dL)	MCV(fl)	HbA2(%)	Mean HbF(%)
SCD	7-8	85-95	2.5-3.5	5
SCD/α-thalassemia	8-10	70-85	3.5-4.5	5
HbS/β^0-thalassemia	8-10	65-75	4-6	9

CONCLUSION

Clinicians of different specialties should understand the relevance of elevated HbF in electrophoretic examinations to correctly direct the diagnostic process. HbF is an important diagnostic parameter in various hematological disorders. Elevated HbF value in adult patients enrolled in our study resulted from Beta^0-Thalassemia, Beta$^+$-Thalassemia, Beta-thalassemia trait, homozygous and heterozygous Drepanocytosis. We found significantly higher HbF values in patients with Drepanocytosis and HbA2</=3.5% compared to those with Drepanocytosis and HbA2>3.5%. HbF values 10-20% suggest HPFH, (δ-β$^-$)-Thalassemia Carriers and Sardinian(δ-β) Thalassemia Heterozygotes. Elevated HbF in combination with HbA2<3.2% requires further investigation since multiple causes may lead to this electrophoretic pattern.

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Conflict of interest disclosure: The authors declare that there is no conflict of interest. The study has been previously presented as a Poster Presentation at 26th Meeting of the Balkan Clinical Laboratory Federation (BCLF) in 2018 and 6th National Congress of the Macedonian Association of Medical Biochemistry and Laboratory Medicine, Skopje, North Macedonia from 3rd to 5thOctober 2018.

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