Hemoglobin variants with electrophoretic mobility similar to Hemoglobin S

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Introduction

Hemoglobinopathies are among the most common inherited diseases around the world and are one of the world's major health problems. They are monogenic diseases of autosomal recessive transmission resulting from mutations affecting the genes responsible for the synthesis of globin chains. Abnormal hemoglobins (Hb), named Hb variants, are caused by structural defects resulting from an altered amino acid sequence in globin chains, being Hb S the more frequent and pathogenic/disease associated.

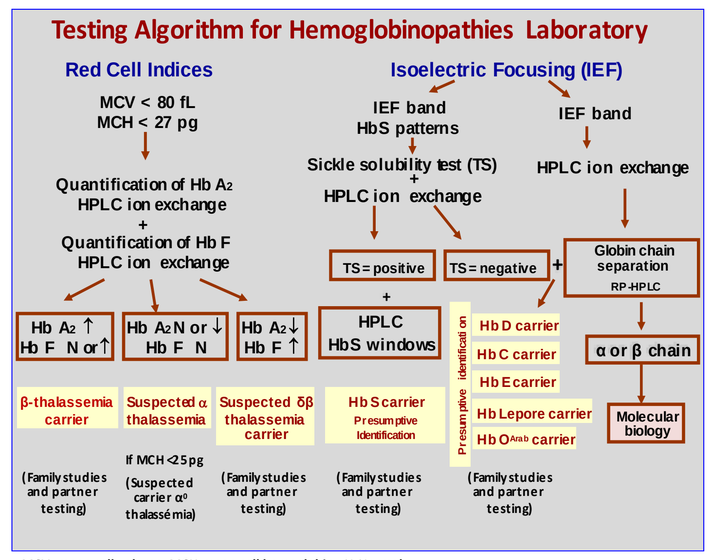
Hemoglobinopathies are unique among all genetic diseases where carriers detection is possible by hematological and biochemical tests. However, identification of Hb variants by these methodologies is often presumptive, based on migration patterns and retention times, and should be based on a minimum of two techniques with different principles. Also, the analytical procedures employed should be able to detect all the common clinically significant hemoglobin variants: Hb S, Hb C, Hb D^{Punjab} , Hb E and Hb O^{Arab}. Nevertheless, in complex cases or when the hematological/biochemical results are unclear, definitive identification requires DNA analysis, mass spectrometry or protein sequencing[1, 2].

Aims

The aim of this work was to identify and characterize Hb variants with mobility similar to Hb S when using common laboratorial methodologies, such as isoelectric focusing and high pressure ion exchange chromatography (HPLC).

Materials and methods

Hemoglobin analysis was performed by isoelectric focusing (IEF) and Ion Exchange High Performance Liquid Chromatography (HPLC). Globin chain variants were classified in alpha or beta type by Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Hb S was confirmed by the Sickle Solubility Test (TS). In order to identify the rare Hb variants, molecular analysis was performed in patient's DNA.



MCV- mean cell volume; MCH-mean cell hemoglobin; N-Normal

Results and discussion

From 2010 to 2016, in the routine practice of our laboratory, 601 cases of variants of Hb were detected with mobility Hb S-like. Amongst them, 433 were confirmed as being Hb S (72.0%). Others hemoglobins also with clinical relevance, Hb D and Hb Lepore, were prevalent, 90 (15.0%) and 61 (10.2%), respectively. The remaining 17 cases were classified as rare (2.8%) and 10 of them were identified by molecular studies as: Hb Maputo, Hb G-Coushatta, Hb Summer Hill, Hb Setif, Hb G Waimanalo, Hb D Iran, Hb Oleander, Hb Ottawa, Hb Etobicoque and Hb Matsue-Oki (Figure 1 and Table 1). Hb Matsue-Oki was found in compound heterozygosity with the $-\alpha^{3.7kb}$-thalassemia deletion.

The presumptive identification of Hb S , Hb D and Hb Lepore was based on migration patterns of IEF and retention times of exchange ionic HPLC of hemoglobins as well as the result of sickle solubility test and chromatographic behavior of reversed phase HPLC globin chains (Figure 2). Rare hemoglobin variants were characterized by our used methodologies and identified by DNA analysis (Figure 3 and 4).

Hemoglobin variants with eletrophoretic mobility similar to Hemoglobin S (2010-2016)

	Hb S	Hb D	Hb Lepore	DNA analysis	Without DNA analysis
No. of cases	433	90	61	10	7
%	72.0	15.0	10.2	1.7	1.1

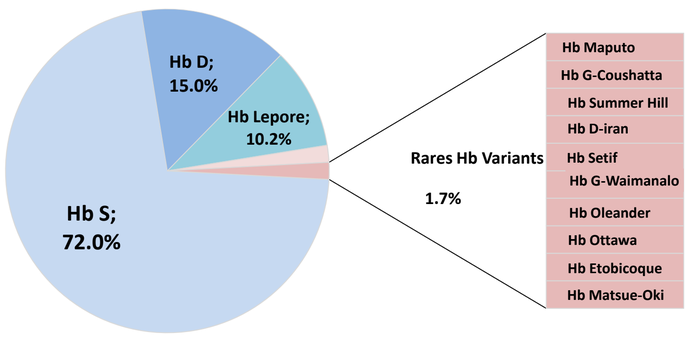


Figure 1- Variants of Hb with mobility Hb S-like detected in our lab during 2000-2016: 433 Hb S, 90 Hb D, 61 Hb Lepore. The remaining 17 cases were classified as rare and 10 of them were identified by molecular studies .

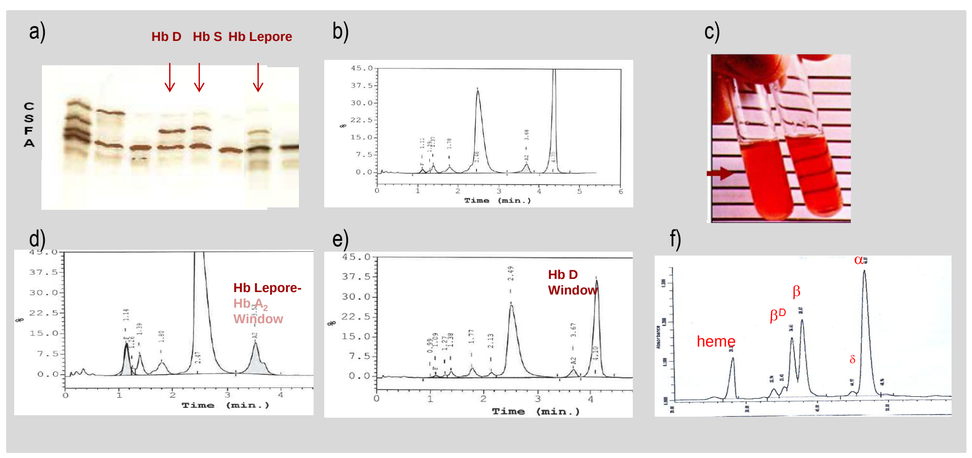


Figure 2- Results of different methodologies for presumptive identification of Hb S, Hb D and Hb Lepore. **a)** Isoelectric focusing migration pattern **b)** Peak on Hb S window (38.3%) by ion iexchange HPLC **c)** Sickle solubility test positive for Hb S **d)** Peak on Hb A_2 window (11.6%) by ion exchange HPLC indicative of Hb Lepore **e)** Peak on Hb D window (37.3%) by ion exchange HPLC **f)** Reversed phase HPLC of globin chains, revealing the presence of β^D chains and normal β chains.

Table 1 - Biochemical and molecular characteristics of rare variants with electrophoretic mobility like HbS

Presumptive Identification										
Isoelectric focusing	**Mobility similar to Hb S**									
Sickle solubility test	**Negative**									
HPLC ion exchange	Peak in the HbD window	Peak in the HbA_2 window	Peak in the HbS window	Peak in the HbA_2 window	Peak in the HbS window or unknown	Peak in the HbS or HbD windows	Peak in the HbS window or unknown	Peak in the HbS window	Peak in the HbS window	Peak in the HbC window
HPLC reversed phase globin chains	β-chain Hb variant	β-chain Hb variant	β-chain Hb variant	β-chain Hb variant	α-chain Hb variant	α-chain Hb variant	α-chain Hb variant	Silent	Silent	Silent
Definitive Identification										
Molecular biology	**Hb Maputo**	**Hb G-Coushatta**	**Hb Summer Hill**	**Hb D-Iran**	**Hb Setif**	**Hb Oleander**	**Hb Etobicoque**	**Hb Ottawa**	**Hb Matsue-Oki**	**Hb G-Waimanalo**
	HBB:c.142G>T	HBB:c.68A>C	HBB:c.157G>C	HBB:c.67G>C	HBA2:c.283G>T	HBA2:c.349G>C	HBA2:c.255C>A	HBA2:c.46G>C	HBA2:c.226G>A	HBA2:c.193G>A

Hb G-Coushatta

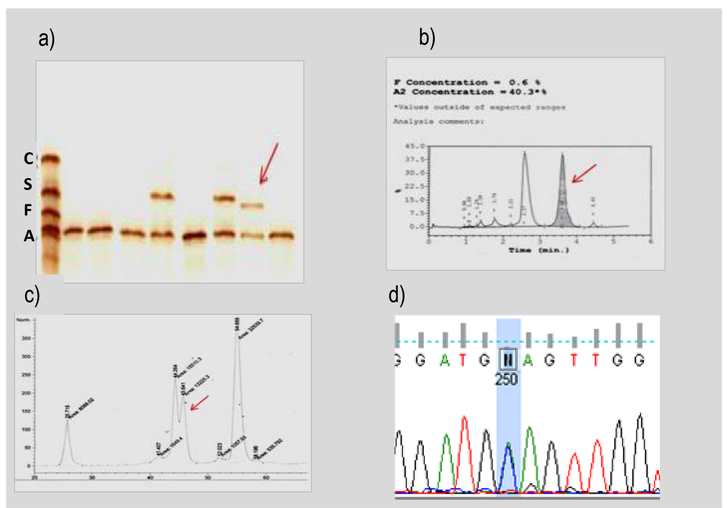


Figure 3- Biochemical characterization and molecular identification of rare beta chain hemoglobin variant, **Hb G-Coushatta a)** Isoelectric focusing migration pattern **b)** Exchange ion chromatographic behaviour showing a peak on Hb A_2 window (40.3%) **c)** Reversed phase HPLC of globin chains, revealing the presence of abnormal β chain **d)** Partial electropherogram of the exon 1-*HBB* gene; heterozygosity for the variant: c.68A>C , p.Glu22Ala.

Hb Matsue-Oki

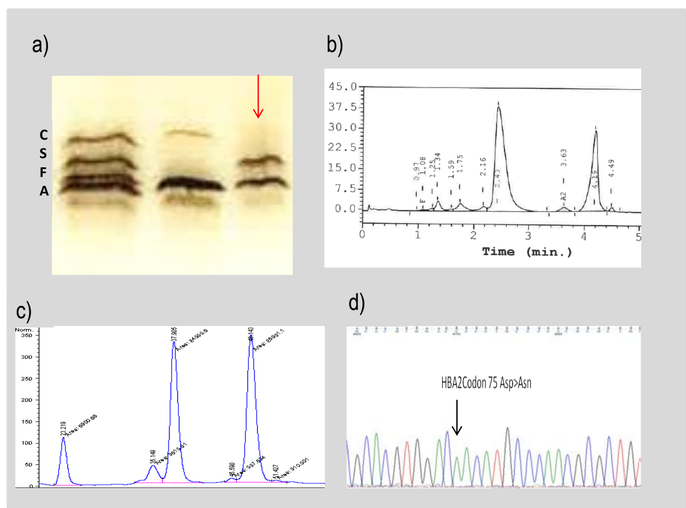


Figure 4- Biochemical characterization and molecular identification of rare alpha chain hemoglobin variant, **Hb Matsue Oki a)** Isoelectric focusing migration pattern **b)** Exchange ion chromatographic behaviour showing a peak on Hb S window (29.4%) **c)** Reversed phase HPLC of globin chains showing a normal elution pattern **d)** Partial electropherogram of the exon 2-*HBA2* gene, hemizygosity for the variant: c.226G>A. p.Asp75Asn.

Conclusion

We can conclude that combining the results obtained by the different biochemical methodologies allow the presumptive identification of the more prevalent variants, namely Hb S, Hb D and Hb Lepore, and direct the molecular study for the definitive identification. This study also revealed that several rare variants have similar mobility as Hb S and, consequently, some safety measures should be applied in order to achieve their accurate identification. A correct laboratorial diagnosis is essential for proper patient's clinical management and genetic counselling.

Bibliography

[1] Ryan K. *et al*. Significant haemoglobinopathies: guidelines for screening and diagnosis. Br. J. Haematol., 2010, 149: 35-49.

[2] Traeger-Synodinos J. *et al*. EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies. Eur. J. Hum. Genet., 2015. 23: 426-37.

(3] Globin Gene Server (http://globin.bx.psu.edu/cgi-bin/hbvar/counter