Five hemoglobin variants detected for the first time by capillary electrophoresis

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This work is dedicated to the memory and in honor of Prof. Renzo Galanello

INTRODUCTION

Hemoglobinopathies are inherited hemoglobin disorders, leading to the synthesis of mutated globin chains or a change in their expression level. Capillary electrophoresis (CE) is one of the methods used to test hemoglobinopathies. Often use in routine, capillary electrophoresis permits fast and precise separation of the hemoglobin fractions, as well as their quantification. Used in our laboratory for five months, capillary electrophoresis allowed us to detect, among others, 5 hemoglobin variants that we report here (Hb Belfast, Hb G-San José, Hb J-Sardegna, Hb Sassari and Hb Shelby) and never described in the literature by this technique.

METHODS

Whole blood samples were analyzed by capillary electrophoresis on CAPILLARYS 2 Flex Piercing (Sebia, France) with the "Hemoglobin(e)" kit, according to the manufacturer's recommendations (1).
Cation-exchange HPLC (Bio-Rad VARIANT II) was used as comparison method.
All variant described here were confirmed by DNA analysis.

RESULTS

Hb Belfast

This variant was found in a 4-years Italian male, with normal hematological parameters, but with Heinz bodies. Mutation was inherited from his father.
Hb Belfast migrates in "S" zone in capillary electrophoresis **(Figure 1)** but can be differentiated from Hb S as migration position is 212 (214 for Hb S), corresponding to the beginning of the zone. In HPLC, Hb Belfast migrates after Hb A2, in "unknown" window.
Hb Belfast is an unstable Beta variant where the Beta 15 Tryptophan is replaced by an Arginine, and shows an increased oxygen affinity. Even if the clinical presentation seems to be normal, Heinz bodies can be found on heterozygous patients.

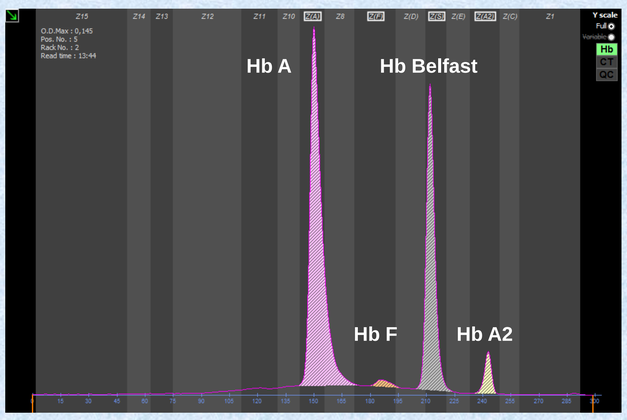

Figure 1: Hb Belfast CE profile.

Hb G-San José

This variant was found in 27-years female from Sardinia. The patient showed normal hematological parameters.
Hb G-San José migrates in "F" zone on capillary electrophoresis **(Figure 2)**. This variant can be suspected because peak level is more important (36%) than those usually found in HPFH (< 30%). Also, the migration position of the variant peak is different from the one observed usually for Hb F peak (second half of the zone when Hb F represents 20 – 30%). In HPLC, this variant elutes immediately after Hb S.
Hb G-San José is a mildly unstable Beta variant where the Beta 7 Glutamate is changed by Glycine. Heterozygous and homozygous patients seem to have normal clinical presentation.

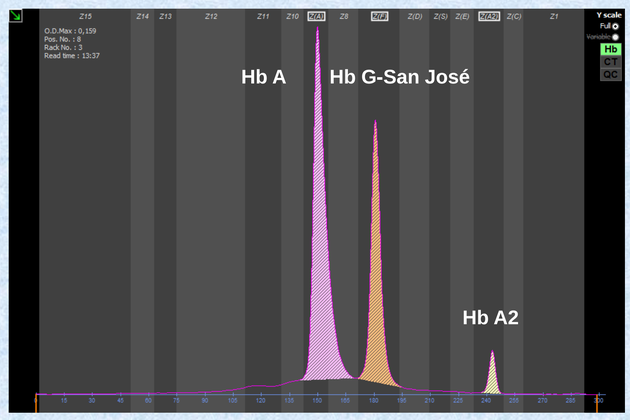

Figure 2: Hb G-San José CE profile.

Hb J-Sardegna

This variant was found in 44-years male from Sardinia, with very slight anemia, not related to hemoglobin variant.
Hb J-Sardegna is an Alpha variant where Alpha 50 Histidine is replaced by Aspartate.
Capillary electrophoresis shows a peak in the zone 12, whereas the minor peak, resulting from $\alpha_2^{var}2/\delta2$, is in the "D" zone near the Hb A2 **(Figure 3)**. Its suggests the presence of an alpha variant.
This variant co-elutes with Hb F in HPLC. Heterozygous patients seem to have normal clinical presentation.

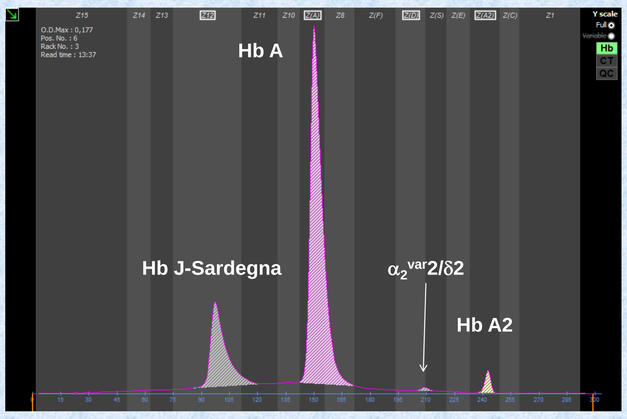

Figure 3: Hb J-Sardegna CE profile.
The minor peak, after HbA2, is an hemoglobin made by α2 globin variant and δ globin chains ($\alpha_2^{Var}2\ \delta2$).

Hb Sassari

This variant was found in 33-years male from Sardinia, showing microcytosis and hypochromia, due to co-inherited non deletional alpha thalassemia.
Hb Sassari migrates in "F" zone on capillary electrophoresis **(Figure 4)** but cannot be confused with Hb F: migration position is different than the Hb F (173, which is very close to the beginning of the zone, against the second half of the zone when Hb F represents 20-30%). The minor peak ($\alpha_1^{Var}2\ \delta2$) located near the Hb A2, suggests the presence of an alpha variant. In HPLC elutes immediately after Hb A2.
Hb Sassari has its Alpha 126 Aspartate replaced by an Histidine and shows and increased oxygen affinity. Clinical presentations found in this patient are those expected for Hb Sassari carriers (mild erythrocytosis).

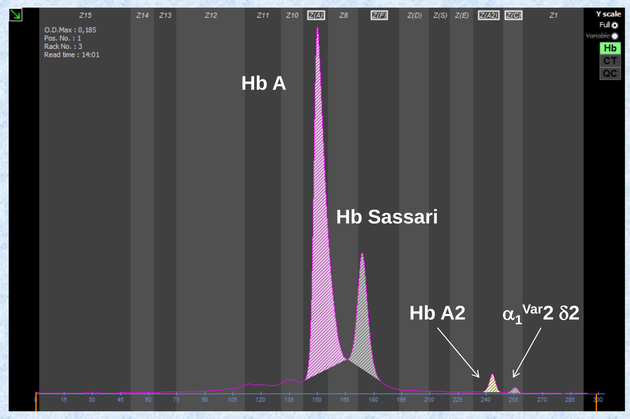

Figure 4: Hb Sassari CE profile.
The minor peak, after HbA2, is an hemoglobin made by α1 globin variant and δ globin chains ($\alpha_1^{Var}2\ \delta2$).

Hb Shelby

This variant was found in a 46-years pregnant female from Sardinia. The patient presented normal hematological parameters.
Hb Shelby migrates in Z8 zone on capillary electrophoresis, between Hb A and Hb F **(Figure 5)**. On HPLC, this variant elutes later, after Hb A2.
Interestingly, during the study, a Hb Shelby sample without Hb A ($\beta^0 39/\beta^{Shelby}$) was also analyzed **(Figure 6)**.
Capillary electrophoresis showed "normal" profile (i.e. without qualitative or quantitative abnormality) but without zone displaying and an alarm, indicating a delay in the profile recentering (that can be explained by an absence of Hb A and/or Hb A2). According to the manufacturer's recommendations, this sample was mixed with normal control and re-analyzed.
The new profile was now recentered: the presumed Hb A peak previously seen is identified between Hb A and Hb F in Z8 zone, at the same migration position (166) than the one observed in the heterozygous patient.
Heterozygous patients seem to have normal clinical presentation.

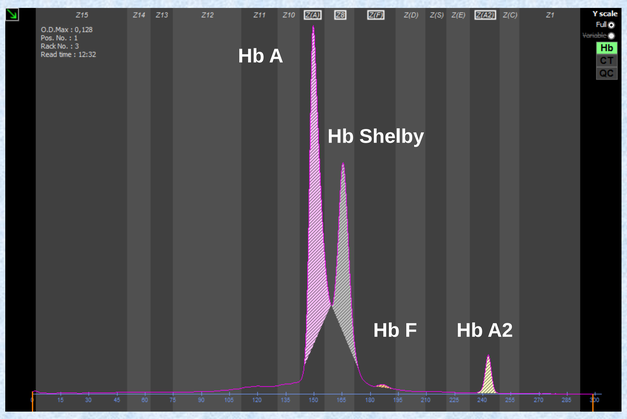

Figure 5: Hb Shelby CE profile.

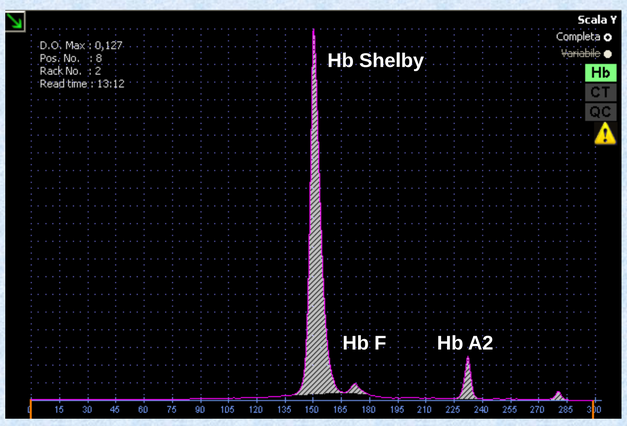

Figure 6: $\beta^0 39/\beta^{Shelby}$ CE profile.

CONCLUSION

Capillary electrophoresis is a powerful technique allowing us to easily identify hemoglobin variants. Here we have shown 5 hemoglobin variants evidenced by capillary electrophoresis and that have never been described to our knowledge by this technique.
Separation is precise, especially if migration position related to the X-Axis is used (2). This technique can be implemented as a first-line screening test, keeping in mind that a confirmatory testing is still required.

REFERENCES

(1) CAPILLARYS HEMOGLOBIN(E) (Ref 2007) , Package insert – SEBIA.
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