

Original article



Hemoglobinopathies in the Constantine region: frequency and difficulties in diagnosing rare variants

Khaled BOUZENDA^{1,2}, Esma MAHDJOUR^{1,2}, Amina LEMRABET¹, Hadjer Zineb ROUABEH¹

¹Department of Hemobiology—Blood Transfusion, Constantine University Hospital. Molecular Biology and Genetics

²Laboratory, Faculty of Medicine, Constantine 3 University. Algeria

ABSTRACT

Aims. Hemoglobinopathies are among the most frequent genetic diseases in the world. Algeria has some endemic areas, mainly for β -thalassemia and sickle-cell anemia, but some rarer variants are sometimes found, whose diagnosis is difficult and may escape interpretation. The aim of this work is to shed light on the cases of rare hemoglobinopathies observed in the Constantine region and the difficulties encountered in their diagnosis. **Material and methods.** This is a descriptive cross-sectional study of 517 capillary hemoglobin electrophoresis profiles on Capillarys and MINICAP Flex Piercing (SEBIA) machines, performed as part of the diagnosis or follow-up of hemoglobinopathies in the hemobiology/blood transfusion department of Constantine University Hospital. Diagnosis is based on clinical findings, blood count results and other biological parameters, family investigation, and hemoglobin studies. **Result.** 71% of the profiles studied were pathological, 26.70% of pathological cases were thalassemic syndromes, of which 26.53% were homozygous β -thalassemia and 67.35% heterozygous, 02 cases of heterozygous δ -thalassemia and 04 cases of heterozygous $\delta\beta$ -thalassemia, 73.3% of pathological profiles were hemoglobinoses, of which 58.74% had a major sickle cell syndrome (SS, S β thal, SC), 24.54% with heterozygous sickle cell disease, 06.69% with heterozygous hemoglobinosis C and 05 homozygous hemoglobinosis C cases, one C/ β thal and 02 C/ α thal cases, 02 composite S/PHHF cases and 02 S/O-Arab cases, 02 composite S/D cases, 02 S/ α thal cases, 03 heterozygous PHHF cases, one heterozygous O-Arab case, one heterozygous hemoglobinosis D case, 03 heterozygous lepore cases and 03 heterozygous hemoglobinosis J cases. **Conclusion:** a precise diagnosis of rare hemoglobin variants is not always obvious, and sometimes only molecular study can provide it.

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CORRESPONDING AUTHOR

Khaled BOUZENDA

khaled_bouzenda@hotmail.com

1. INTRODUCTION

Hemoglobinopathies are among the most common genetic diseases worldwide. As a result of various migratory flows in recent decades, their worldwide distribution has become cosmopolitan [4]. The pathological consequences of a mutational event in the genes encoding globin chains are usually of two types. They can affect either the structure of hemoglobin, in which case the anomaly is qualitative, or the mechanisms of its synthesis, in which case the anomaly is quantitative [7-8]. In the first case, we speak of abnormal hemoglobin or hemoglobinosis,

as in the case of hemoglobin S, which is responsible for sickle cell disease. The second is called thalassemia, a term that encompasses a heterogeneous group of hematological disorders characterized by the absence or reduced synthesis of one or more globin chains. There are also mixed anomalies and associations [9,12].

Algeria has certain areas endemic mainly for β -thalassemia and sickle-cell anemia, the management of which is still a public health problem, but certain rarer hemoglobin variants are

sometimes found in this region, the diagnosis of which is difficult and may escape interpretation [13-15].

The aim of our work is to search for and detect hemoglobinopathies present in the population of the Constantine region, essentially rare hemoglobins, and to report the main biological aspects, enabling them to be better characterized to make an accurate diagnosis and to prevent severe homozygous or heterozygous composite forms by providing well-adapted genetic counseling [16-18].

2. METHODS

This is a descriptive cross-sectional study of 517 capillary hemoglobin electrophoresis profiles performed on Capillarys and MINICAP Flex Piercing (SEBIA) automated systems as part of the diagnosis or follow-up of hemoglobinopathies in the hemobiology/blood transfusion department of Constantine University Hospital.

For each patient, an information sheet is completed, containing the information needed to interpret the results, i.e. demographic data, clinical information and results of biological explorations. Whole blood is drawn aseptically from the elbow vein and collected in a 05 ml tube with EDTA anticoagulant. Samples are taken either at the blood collection unit of the hemobiology/blood transfusion department of Constantine University Hospital, or at the level of the health care services concerned, and then forwarded to the hemobiology department for analysis. This sample will be used both to perform a complete blood count (CBC), supplemented by a blood smear if necessary, and to perform capillary Hb electrophoresis on fresh blood or blood stored at +4°C within a maximum of 7 days. The electrophoretic profiles appear in the PHORESIS software, supplied by SEBIA, where they are visually interpreted to detect any anomalies, and a relative quantification of the different hemoglobin fractions is systematically carried out by the automatons.

All electrophoreses prescribed for the investigation or follow-up of hemoglobinopathies are included in the study, as are the electrophoreses of family members of patients called in for family investigations. Non-compliant samples, incomplete or incorrectly filled in information sheets by the prescribing healthcare service, and redundant samples of control electrophoreses for the same patient are excluded.

Interpretation of profile results and diagnosis of hemoglobinopathies are based on clinical findings, blood count and other biological parameters, family history, ethnic origin and the result of hemoglobin studies by capillary electrophoresis [2,5,10]. the anonymity of the patients was respected and the study is in accordance with the Helsinki Declaration.

3. RESULTS

Most hemoglobin electrophoresis prescriptions came from outpatient consultations, accounting for 37.72% of cases, and the hospital department that prescribed this test the most was the hematology department, accounting for 27.85% of cases. The pediatrics department represented 8.70% of prescriptions, 18.96% of cases were conducted as part of family investigations, and the remaining 6.77% of prescriptions were received from various hospital departments. In our population, there was a slight predominance of women (57.06% of cases), with a sex ratio of 1.33:1. Most prescriptions (92.26% of cases) were made with the aim of establishing or confirming the diagnosis of a hemoglobinopathy. The remaining 7.74% were intended for electrophoresis in patients with known major sickle cell syndrome, to monitor HbS levels after exchange transfusions in preparation for a surgical intervention or a scheduled delivery.

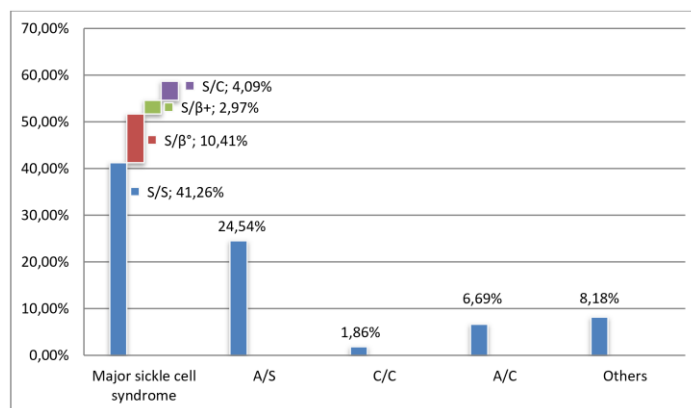


Figure 1. Distribution of hemoglobinotypes in our study population.

Among the 517 profiles studied, 367 cases (71%) had pathological profiles and 150 cases (29%) had normal profiles. The study of hemogram abnormalities in subjects with hemoglobinopathies showed that 24.29% of subjects had a normal hemogram, and the most frequent blood count abnormality was microcytic anemia with 23.16% of hemoglobinopathy cases. The other abnormalities observed in the hemogram were respectively: microcytic pseudopolycythemia (17.71%), hypochromic microcytic anemia (16.89%), isolated anemia (12.26%), isolated microcytosis (5.18%), and hypochromic anemia (0.55%).

Among the pathological profiles in our study population, 26.70% of cases were thalassemic syndromes, of which 26.53% were homozygous β-thalassemias and 67.35% of cases were heterozygous β-thalassemias. There were two cases of heterozygous δ-thalassemia and 04 cases of heterozygous δβ-thalassemia, while 73.3% of pathological profiles were hemoglobinoses (Figure 1), of which 58.73% presented a major sickle cell syndrome (S/S, S/β-thal, S/C), 24.54% heterozygous

sickle cell disease, 06.69% heterozygous hemoglobinosis C and 05 cases of homozygous hemoglobinosis C.

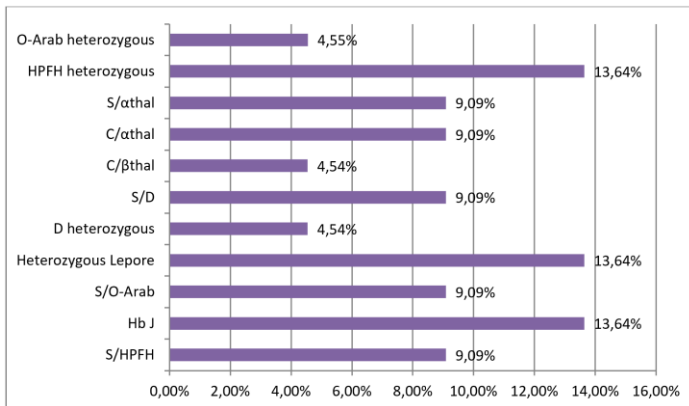


Figure 2. Distribution of rare hemoglobins in our study population

At the end of this study, 22 subjects (8.18%) were identified as having rare hemoglobin variants, either heterozygous or composite heterozygous (figure 2). one C/βthal case and 02 C/αthal cases, 02 heterozygous composite S/HPFH cases (figure 3) and 02 S/O-arab cases, 02 composite S/hemoglobinosis D cases, 02 S/αthal cases, 03 heterozygous hereditary persistence of fetal hemoglobin (HPFH) cases, one heterozygous O-arab case, one heterozygous hemoglobinosis D case, 03 heterozygous lepore cases (figure 4), and 03 heterozygous hemoglobinosis J cases (figure 5).

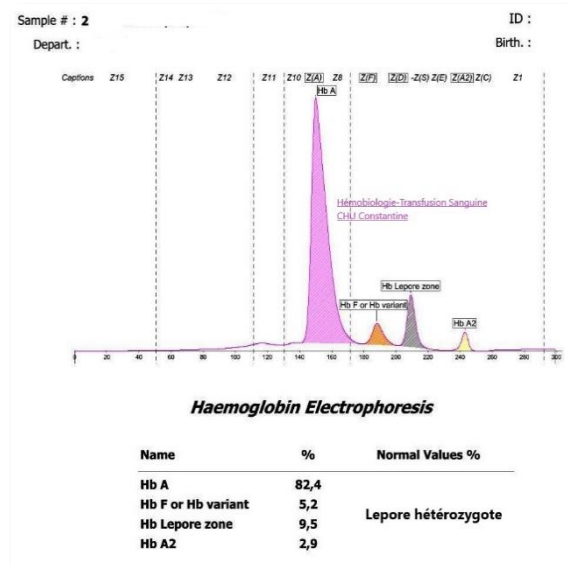


Figure 4. Profile of a patient with heterozygous Lepore hemoglobinosis

4. DISCUSSION

Most of our prescriptions come from non-hospitalized patients, either following outpatient consultations (37.72%) to explore an obvious anemic syndrome or an abnormality observed on a blood count performed in different contexts, or as part of a family investigation (18.96%) carried out to confirm the diagnosis of a hemoglobinopathy in a patient by demonstrating the abnormality in family members. Otherwise, the vast majority of prescriptions were referred to the hematology (27.85%) and pediatrics (08.70%) departments, as they specialize in the management of this type of pathology.

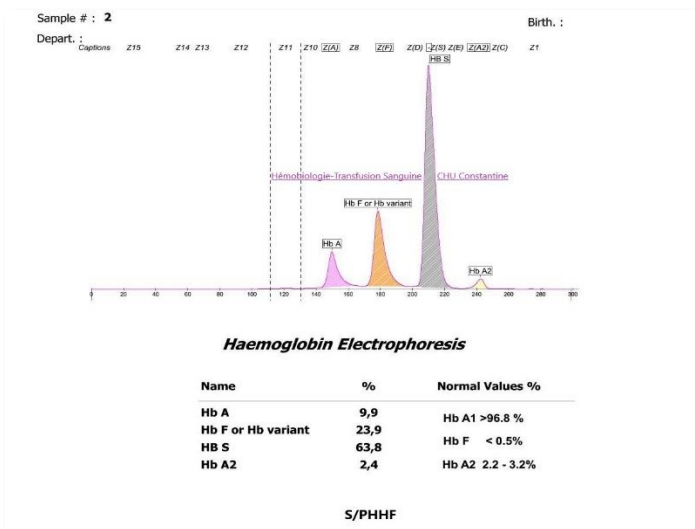


Figure 3. Profile of a composite heterozygous S/HPFH patient

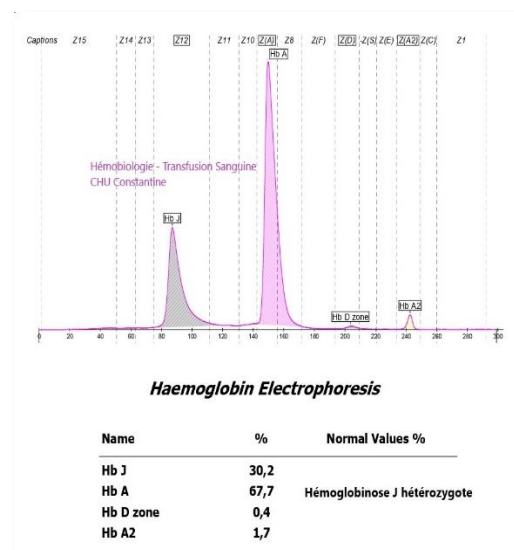


Figure 5. Profile of a patient with heterozygous hemoglobinosis J

In our population, there is a slight predominance of females, with a sex ratio of 1.33 F/H, unlike the study by Belhadi et al 2011. [3] from the Batna region, where the predominance was male. This is not related to the distribution of these pathologies, whose transmission is autosomal recessive and unrelated to gender, and this difference can be explained in part by the fact that women are screened much more frequently than men, because they are more regularly monitored, particularly during pregnancy.

Of the 517 profiles studied, 367 (71.02%) were pathological, while 28.98% were normal, either in patients with other pathologies that were the cause of the clinico-biological disturbances that prompted Hb electrophoresis or in healthy subjects investigated during family surveys. 24.25% of cases with hemoglobinopathies had a normal hemogram, while the remainder had disturbed hemograms, which in some cases were the main reason for hemoglobin studies. The results of the hemograms were in perfect agreement with the hemoglobinopathies observed in the patients, and were used as elements of orientation and confirmation of the diagnosis.

In addition, our study showed a predominance of hemoglobinoses (qualitative hemoglobin anomalies) with a rate of 73.3%, compared with thalassemic syndromes (quantitative hemoglobin anomalies) (26.7%), and this is consistent with the study by Dahmani F et al [6], with WHO data [11], and with the study by Belhadi K et al in the Batna region [3]. However, our results are not consistent with those of Abbassen N et al, in the Sétif region [1], where thalassemic syndromes were more frequent than hemoglobinoses. This observation can be explained by the fact that Algeria has regions endemic for hemoglobinopathies, and the distribution of these pathologies varies from one region to another, as do the genetic anomalies that predominate. Heterozygous β -thalassemias are the most frequent thalassemic syndromes in our study (67.35%). These cases are generally asymptomatic with a mildly disturbed blood count, but still require good genetic counseling to prevent the most severe forms. Homozygous SS sickle cell disease is the most common hemoglobinosis in our study (41.26%). Double heterozygous S/ β^0 thalassemia (10.41%), S/ β^+ thalassemia (2.97%), and S/C (4.09%) hemoglobinoses, although less frequent than homozygous S/S, are not uncommon and are the cause of a major sickle cell syndrome as severe as homozygous sickle cell disease, and whose management is just as restrictive. In our study, heterozygous hemoglobinosis C accounted for 6.69% of hemoglobinosis cases, and 5 homozygous C/C cases were detected, demonstrating that this hemoglobinopathy is not rare in the Constantine region. Although rare hemoglobin variants represent a low proportion in our study (figure 1), a better understanding of the pathophysiological mechanisms and clinico-biological characteristics of these rare forms is crucial for accurate diagnosis, effective management and appropriate genetic counseling, although the difficulty of conducting a proper family investigation due to the unavailability of one of the parents because of death or estrangement, and the lack of means

for molecular study, make diagnosis and confirmation of these variants very difficult.

5. CONCLUSION

The diagnosis of hemoglobinopathies is based on several parameters, such as the blood count, hemoglobin studies and family investigations. Nevertheless, in certain situations, even when all the above-mentioned elements are present, it is very difficult to make a precise diagnosis. This is essentially the case for rare hemoglobin variants, which are increasingly being highlighted, and for which a molecular study is necessary in order to remove ambiguity in the interpretation of the patient's clinico-biological observations, but also for better management, and genetic counseling well adapted to each situation will thus make it possible to prevent and limit the occurrence of the most serious forms of these pathologies.

Competing interests: The authors declare that they have no competing interest.

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