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Epidemiological, Clinical and Biological Profile of O Arab Hemoglobinosis: Experience of the Biochemistry Toxicology Department of HMIMV

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Abstract **Original Research Article**

Background: Hemoglobinopathies (HbPs) are inherited disorders of red blood cells caused by abnormalities in the globin chains of hemoglobin (Hb). Hemoglobin O-Arab is a specific variant with a mutation in the β-chain. These disorders range from asymptomatic to severe and represent a significant public health issue. ***Methods:*** A retrospective study was conducted over 10 years (February 2014 to April 2024) in the Biochemistry-Toxicology Department of HMIMV in Rabat. Hemoglobin variants were detected using HPLC and capillary electrophoresis. Data on socio-epidemiological, clinical, and biological aspects were collected from patient follow-up forms. ***Results:*** of 17,843 hemoglobin studies, 1,154 hemoglobinopathies (5.4%) were identified, with 63 cases (0.35%) of hemoglobin O-Arab: 58 heterozygous, 4 homozygous, and 1 composite heterozygous. Most cases were detected incidentally during HbA1c testing. Homozygous cases showed anemia, while heterozygous cases showed no significant abnormalities. The composite heterozygous case had severe clinical complication. ***Conclusions:*** Hemoglobinopathies, including hemoglobin O-Arab, are common genetic disorders. Advanced diagnostic techniques like capillary electrophoresis have improved detection, but multiple methods are still needed for accurate diagnosis. Effective collaboration between clinicians and laboratories is crucial for managing these disorders.

Keywords: Hemoglobin O-Arab, Hemoglobinopathies, Genetic disorders, Capillary electrophoresis.

INTRODUCTION

Hemoglobin (Hb), the main component of the red blood cell (RBC), is a ferroproto-porphyrin heteroprotein. It is formed in the cytosol from the union of four heme molecules and four globin polypeptide chains identical in pairs.

Hemoglobinopathies (HbP) are inherited disorders of the red blood cell resulting from structural or synthetic abnormalities in the globin chains. The pathology is heterogeneous at clinical, biological and molecular levels, ranging from totally asymptomatic to the most severe forms. The frequency and distribution of hemoglobinopathies worldwide, and the still limited means of treatment, make these anomalies a real public health problem. Hemoglobinopathies can occur in a number of genetically varied forms. These may be heterozygous, homozygous, double heterozygous or a double combination of different hemoglobinopathies [1].

Hemoglobin OArab (Hb OArab) is an abnormal hemoglobin characterized by the substitution of lysine for glutamic acid at position 121 of the b-globin chain. The electrophoretic mobility of Hb O-Arab is similar to Hb C, Hb E, and Hb A2 on cellulose acetate at alkaline pH. In contrast, Hb OArab migrates close to Hb S on citrate agar at acidic pH [2].

The medical biology laboratory, in close collaboration with clinicians, plays an undeniable role in the management of this type of pathology. Biological investigations are essential for the precise identification of abnormal Hb, and call on a range of techniques required for the typing of different Hb variants and the quantification of normal or non-normal Hb. This forms the basis for reliable diagnosis of HbP, which will enable not only appropriate management of affected patients, but also assessment of the risk of transmission of these diseases to the offspring of Hb-abnormal parents.

The routine use of capillary electrophoresis has revolutionized the way in which this diagnosis is made. The aim of this study is to report the socio-epidemiological, clinical and biological data on O-arabhemoglobinoses in a series of 63 cases diagnosed in the biochemistry-toxicology department of the HMIMV in Rabat over a period of 10 years.

MATERIALS AND METHODS

This is a retrospective study conducted in the Biochemistry-Toxicology Department of the HMIMV in Rabat over a 10-year period from February 2014 to April 2024.

The detection of the variant was primarily incidental in most patients, identified through HPLC methods during HbA1c testing. In other cases, detection was done using capillary electrophoresis on the CAPILLARYS 3 TERA analyzer (Sebia). Socio-epidemiological, clinical, and biological data were collected using a follow-up form that accompanies all hemoglobin study requests. The form includes the patient's name, age, gender, origin, clinical data, results of biological examinations, and treatments (including recent transfusions or treatments).

Blood samples were collected via venipuncture into EDTA tubes. Samples must be delivered to the laboratory within 12 hours. The sample should be fresh or, if necessary, refrigerated at 4°C for a maximum of seven days. Freezing is to be avoided to prevent hemolysis. It is important to note that the search for an Hb anomaly should be performed at least three months after any blood transfusion and after ruling out iron deficiency (preferably with normal ferritin levels).

Hb Anomaly Detection According to the Nomenclature of Medical Biology Acts (NABM), the detection of an Hb anomaly must include a minimum of three distinct phenotypic tests. These must include at least one electrophoretic technique and two other tests tailored to the diagnostic needs. Among the three techniques used, one must be quantitative to precisely measure minor fractions (HbA2 and HbF). Therefore, only HPLC and capillary electrophoresis (CE) are acceptable [3].

The Biochemistry-Toxicology laboratory employs a complementary set of examinations and tools for Hb study. Detection of the variant in most patients was incidental during HbA1c testing by HPLC. For others, it was directly identified using capillary electrophoresis on the CAPILLARYS 3 TERA analyzer (Sebia). Subsequently, agarose gel electrophoresis at acidic pH on Hydrasys® (Sebia®) is performed to distinguish the C variant from the O-Arab variant for diagnostic guidance.

To correctly interpret the results of Hb studies, the Biochemistry-Toxicology laboratory of HMIMV relies on a combination of epidemiological (ethno-geographic origin, age, family history, and parental consanguinity), clinical (clinical signs, pathological history, recent transfusions), hematological (complete blood count with erythrocyte indices [MCV, MCH] to determine the presence and nature of anemia [hypochromic, microcytic], regenerative or non-regenerative [reticulocyte count], blood smear to note morphological anomalies of erythrocytes [anisopoikilocytosis, target cells]), and biochemical (Hb electrophoresis, HPLC, iron status, hemolysis profile) arguments. This comprehensive approach facilitates diagnosis in the majority of cases. In the presence of ambiguous or complex profiles, molecular studies are recommended to type the Hb anomaly, as recommended by the NABM.

RESULTS

Over the 10-year period, 17843 hemoglobin studies were conducted in the laboratory, and 1154 hemoglobinopathies were detected (5.4%). Among these studies, 63 patients (0.35%) were found to carry O-Arab hemoglobin. These patients are distributed as follows: heterozygous A/O (n=58, 92.1%), homozygous O/O (n=4, 6.3%), and composite heterozygous S/O-Arab (n=1, 1.4%).

We note the diversity of clinical services from which the cases studied come. Notably, nearly 82% of the HbP cases were referred by internal medicine, clinical hematology, and pediatrics departments. The study cohort included 27 male subjects (42%) and 36 female subjects (58%), with a sex ratio of 0.75. The mean age was 53 years, with extremes ranging from 18 to 84 years.

The majority of cases were discovered incidentally by the detection of a variant in the HBA1C HPLC assay (75.3%). The remaining cases were identified through hemoglobin electrophoresis requested for anemia evaluation (19.8%) or splenomegaly investigation (4.9%). Homozygous forms (O/O) are often discovered by the presence of microcytic hypochromic anemia (83%). For the composite heterozygous form (O/S), discovery is based on a family screening. Heterozygous forms (A/O) are often discovered incidentally (97.9%).

The hemograms of homozygous subjects were characterized by the presence of hypochromic microcytic anemia (average Hb=10.9 g/dL), an increased reticulocyte count (>120G/L), and the presence of target cells in the blood smear. In contrast, heterozygous A/O-Arab subjects showed no quantitative or morphological abnormalities.

For the S/O-Arab form, it was characterized by normocytic normochromic anemia (Hb=5.8 g/dL; MCV=91.2 fl; MCH=29.1 pg). The blood smear showed target cells and sickle cells. Hemolysis parameters were

disturbed only in the heterozygous S/O-Arab patient, with elevated conjugated bilirubin, LDH, and decreased haptoglobin levels.

In heterozygous A/O-Arab cases, the average hemoglobin O level was 39.6% ± 3.3, with hemoglobin A at 61.1% ± 5.3, hemoglobin A2 at 0.96% ± 0.89, and hemoglobin F at 0.48% ± 0.30. In homozygous O/O cases, the average hemoglobin O level was 97.1% ± 2.3, hemoglobin A2 at 0.48% ± 0.30, hemoglobin F at 3.00% ± 1.30, and no HbA. For the composite heterozygous O/S form, the values of the different hemoglobin fractions were 92% for fraction A, 5% for fraction O-Arab + A2, 2.3% for fraction S, and 0.3% for fraction F.

The progression of hemoglobin O disease is related to its clinical form. The heterozygous form (A/O) is asymptomatic and inapparent. The homozygous O-Arab/O-Arab form is well-tolerated.

The evolution of the heterozygous S/O composite subject in our study was marked by sickle cell nephropathya major complication of sickle cell disease. It manifested as glomerulopathy, proteinuria and hematuria, leading to end-stage renal disease (ESRD). The patient was placed on peritoneal dialysis and treated with erythropoietin ARANESP 30µg/ every 2 weeks, with a blood transfusion of 2 packed red blood cells every two months. She deceased at the age of 26 due to worsening renal failure.

DISCUSSION

Worldwide, 7% of the population are carriers of hemoglobinopathies, making them the most common monogenic diseases and one of the major health issues worldwide. Morocco, due to its geographical location, ethnic origins, and high frequency of consanguineous marriages, has a relatively high prevalence of hemoglobin disorders [4].

According to our survey, the prevalence of hemoglobin O-Arab is around 5.2% (n=63) compared to all hemoglobinopathies (1,057 patients). This prevalence is slightly higher compared to a series done in a private laboratory. The sex ratio is 0.75, with a slight female predominance, which is roughly in line with the study of 20 patients from north-western Tunisia [5] with a sex ratio of 0.53 (7 men and 13 women).

As mentioned in the results section, the average age at diagnosis is 53 years, ranging from 18 to 84 years. Our age distribution shows a clear predominance of adults compared to children. The late age of diagnosis is due to the fact that this variant is often asymptomatic in its heterozygous and homozygous forms. However, in the composite form (O/S), the age at diagnosis is earlier due to its severity. In this study, the diagnosis of Hb O-Arab was made either incidentally during the management of another pathology or in the context of clinical and biological signs suggestive of an Hb anomaly.

A study in Côte d'Ivoire and West Africa involving 44 cases of Hb O-Arab [6], divided into three phenotypes (A/O Arab, C/O Arab, and S/O Arab), confirmed that the A/O Arab heterozygous form is completely asymptomatic. Our findings indicate that A/O heterozygosity is well-tolerated without clinical or hematological consequences.

In the Tunisian series by Hafsia [4], homozygous individuals exhibited no symptoms upon admission. However, anemia and splenomegaly were observed in 100% and 15% of homozygous cases, respectively. Similarly, the homozygous form in our series resulted in moderate, well-tolerated hemolytic anemia (average Hb=10.9 g/dL).

The S/O-Arab association causes a rare clinical syndrome similar to SS sickle cell disease [7]. Hb O-Arab stabilizes the intracellular polymerization of HbS, leading to irreversible sickling of erythrocytes, presenting a more severe clinical picture, including hemolytic anemia and vaso-occlusive complications [8]. In our study, the S/O-Arab association represents a severe clinical form, with a hemogram showing microcytic hypochromic anemia (Hb=5.8 g/dL), increased reticulocyte count (>120G/L), target cells in the blood smear, and hemolysis profile abnormalities, including elevated conjugated bilirubin and LDH, and decreased haptoglobin.

A cohort of 13 African American patients with Hb S/O-Arab [2] demonstrated hemolytic anemia with a median Hb concentration of 8.7 g/dL and a reticulocyte count of 5.8%. Blood smears from all patients revealed crescent or sickle-shaped erythrocytes, similar to those seen in Hb SS. Each patient experienced sickle cell events with significant morbidity despite a median age of only 15 years. Five of the 13 patients developed gallstones requiring cholecystectomy, and four developed sickle cell nephropathy, with two cases progressing to chronic kidney disease. Three childhood deaths occurred: two from pneumococcal sepsis and meningitis between 5 and 10 years, and one from acute chest syndrome. In our study, the patient with Hb S/O-Arab died at age 26 due to end-stage renal disease.

A study conducted in Tunisia on 20 cases [3] found that the homozygous form was not very symptomatic. The heterozygous composite Hb O/beta-thalassemia form was more severe, characterized by mild thalassemia with moderate microcytic hypochromic anemia (Hb = 8.8 g/dL). This form was often complicated by thrombocytopenia due to hypersplenism in 40% of cases.

CONCLUSION

Hemoglobinopathies are among the most common genetic disorders globally and in Morocco. As we have observed, capillary electrophoresis has significantly advanced the diagnosis of many hemoglobin variants, including hemoglobin O-Arab, due to its simplicity, speed, and excellent resolution. It is a powerful technique, comparable to HPLC, but with lower operating costs.

However, some variants remain undetectable by this method, underscoring the need for multiple techniques to provide a reliable diagnosis. Additionally, the importance of clinical, ethnic, biological, and hematological parameters, and the quality of clinico-biological dialogue, cannot be overstated in any hemoglobin study.

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