Pattern of Hemoglobinopathies: A Cross-sectional Study in North of Morocco

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Research

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Abstract

Background: Hemoglobinopathies are the most frequent widely spread genetic disorders. In Morocco, epidemiological and clinical data are scarce. The present study aims to determine the spectrum and geographic distribution of hemoglobinopathies in Larache province, North of Morocco.

Methods: A retrospective cross-sectional study was conducted from January 2015 to December 2018 at the provincial hospital of Larache city. All patients’ records having a hemoglobinopathy were analyzed. Background data (age, gender, and origin) of each case were analyzed.

Results: Our study showed an overall frequency of hemoglobinopathies of 3.6%. Sickle cell trait was registered to be the most common disorder in our studied population (42.3%), followed by β-thalassemia Trait (20.2%), sickle cell disease (19.8%), major β-thalassemia (9.8%) and sickle/β-thalassemia (8%). The average age of subjects with hemoglobin disorders was 17.5 years. The majority of the patients (66.5%) were less than 20 years old. A disparity in the diseases geographic distribution was observed, 74.6% of patients came from rural areas.

Conclusions: The present study is the first of its kind offering comprehensive data on hemoglobinopathies pattern in Northern Morocco. Our work may lay the foundation for screening programs for better prevention and coverage.

Background

Hemoglobinopathies are a group of inherited monogenic hemoglobin disorders. They are recessively transmitted and fall into two main groups: Sickle Cell Diseases and Thalassemias. The clinical manifestation of these diseases varies significantly from a patient to another (1, 2). Due to populations mobility hemoglobinopathies have become the most spread worldwide genetic diseases (3). The World Health Organization (WHO) reported that at least 5.2% of the world population carries a significant variant and over 330,000 affected infants are born annually (83% Sickle Cell Disorders, 17% Thalassemias) (4). In Morocco, these diseases are recognized to be a significant public health problem. In 1984, the frequency of the heterozygous population was estimated at 6.5%, suggesting the existence of a total number of 30,000 cases of major forms of Thalassemia and Sickle Cell Diseases (5). To date, a national registry of patients still not exist and the exact diseases spectrum remains unknown.

Scarcie studies reported the Northwest region as a geographic hotspot for hemoglobinopathies (6, 7). The present work aims to determine the diseases patterns, geographic distribution, and their epidemiological characteristics in Larache province. This would be highly useful to design a targeted and cost-effective regional prevention and control plan.

Methods

A total of 12000 patients were analyzed from January 2015 to December 2018 in a cross-sectional, hospital-based retrospective study conducted in the provincial hospital Princess Lalla Meriem of Larache city, Northern Morocco (Fig. 1). This Hospital is the unique health institution in the province; it provides medical care to all the local population, and offers diagnosis and medical coverage to all hemoglobinopathies patients in the entire region.

Our study recruited the confirmed hemoglobinopathies patients out of the studied medical records. All the information (age, origin, pathological history, clinical features and laboratory data) was registered.

The diagnosis of hemoglobinopathies was confirmed by complete blood count (CBC) using the automated cell counter SYSMEX XT-1800 (Kobe, Japan), and Hemoglobin electrophoresis using the Minicap system (Sebia, France).

A mean corpuscular volume (MCV) < 80 fL and/or mean corpuscular hemoglobin (MCH) < 27 pg and elevated values of hemoglobin A2 fraction (>3.5%) were taken as cut off point for determining the β-thalassemia trait. The diagnosis of sickle cell/β-thalassemia was based on elevated Hb A2 levels (>3.5%) in the presence of Hb S fraction (8).

The overall observed frequencies of sickle cell diseases and β-thalassemia were defined by analyzing hemoglobin variant. The observed frequencies were calculated using Hardy-Weinberg law (9).

Statistical analysis was carried out using SPSS statistical software V.22 (Chicago, IL, USA). The quantitative variables were expressed on mean and standard deviation and were done with Student T-test. The qualitative variables in number and percentage and were compared using the Chi-square test (such a result is considered statistically significant iff P-value < 0.05).

Results

Our study identified 440 patients with hemoglobinopathies in the 12000 studied subjects. (Table 1). The overall frequency of hemoglobinopathies was found at 3.6% with an average of nine new individuals per month. Sex ratio M/F was found 1.01 and the average age was 17.5 years ranging
from two months to 70 years old.

Out of the 440 hemoglobin disorder's patients, βS mutation was detected in 308 subjects (70%). 186 of them (42.3%) carried the heterozygous form βA/βS, (sex ratio: 0.95), 87 patients (19.8%) were homozygous βS/βS (sex ratio: 1.02) and 35 patients (8%) (Sex ratio: 1.18) were detected to have an Hb S/β-thalassemia. SCT subjects showed an average of 38.17 ± 4.11% of Hb S with a slight decrease in the Hb A. SCA patients were characterized by the presence of Hb S fraction with an average of 62.96 ± 13.4%, a minor expression of Hb A and a high-level of fetal hemoglobin Hb F (mean value: 17.98 ± 9.39%). The double heterozygosity Hb S/β-thalassemia showed elevated rates of Hb S at 69.39 ± 17.78%, Hb A2 rates of 5.24 ± 1.88% and decreased Hb F levels.

Major β-Thalassemia patients (9.8%) (Sex ratio: 1.38) registered the lowest hemoglobin average and microcytic hypochromic anemia. A significant decrease was observed in Hb A (20.35 ± 14.4%), an increase in Hb A2 and high increase in Hb F levels.

β-Thalassemia trait was present among 89 subjects (20.2%) (Sex ratio: 1.06). They showed milder anemia and a slight decrease of Hb A associated with an increase of both Hb A2 and Hb F compared to normal subjects.

Hematological parameters and hemoglobin variants levels are presented in Table 1.

Regarding patients gender, most of the SCA patients (91/186 patients), major β-Thalassemia (25/43) and the sickle/β-thalassemia subjects (19/35) were males. However, the majority of SCT (42/78) and all four β-thalassemia trait patients were females (Table 1). Out of the 440 patients, 222 (50.5%) were males and 218 (49.5%) were females. This difference was found statistically not significant (χ2 = 0.125, P-value > 0.05).

### Table 1

| Hb pattern          | Total N(%) | Female N | Male N | Mean age | Hb (g/dL) | Hb A2% | Hb A% | Hb F% | Hb S% | RBC (x 106/µL) | Hct% | MCV (fL) | MCH (pg) | MCHC (g/dL) |
|---------------------|------------|----------|--------|----------|-----------|--------|-------|-------|-------|----------------|------|----------|----------|-------------|
| Sickle Cell Trait   | 186 (42.3)| 91       | 95     | 22.5     | 10.92 ± 1.71 | 3.23 ± 0.43 | 56.6 ± 5.35 | 10.08 ± 2.16 | 38.17 ± 4.11 | 4.63 ± 0.65 | 35.95 ± 4.9 | 70.48 ± 5.03 | 26.12 ± 3.18 | 33.18 ± 1.25 |
| Sickle Cell Anemia  | 89 (20.2)| 43       | 46     | 11.7     | 7.86 ± 1.29 | 3.47 ± 1.4 | 5.76 ± 2.38 | 17.98 ± 9.39 | 62.96 ± 13.4 | 2.73 ± 0.78 | 23.53 ± 4.06 | 75.6 ± 10.6 | 27 ± 4.38 | 33.64 ± 1.7  |
| β-Thal trait        | 87 (19.8)| 44       | 43     | 19.8     | 11.16 ± 1.56 | 5.46 ± 0.56 | 83.43 ± 1.8 | 1.78 ± 0.6 | 0 | 5.59 ± 1.05 | 34.5 ± 4.6 | 63.91 ± 6.64 | 20.4 ± 3.36 | 31.94 ± 0.79 |
| β-Thal Major        | 43 (9.8)| 25       | 18     | 11.9     | 6.1 ± 2.91 | 5.1 ± 1.08 | 20.35 ± 6.4 | 74.55 ± 8.6 | 0 | 3.89 ± 1.5 | 20.8 ± 4.95 | 65.9 ± 9.1 | 24.05 ± 4.17 | 31.55 ± 2.92 |
| Sickle Cell S/β-     | 35 (8)    | 19       | 16     | 12.9     | 7.45 ± 1.74 | 5.24 ± 1.88 | 12.28 ± 5.19 | 8.09 ± 5.08 | 50.39 ± 17.78 | 3.15 ± 0.67 | 22.68 ± 2.51 | 73.71 ± 7.6 | 24.29 ± 2.34 | 32.9 ± 0.88  |

Values mentioned are mean ± standard deviation. Hb-Hemoglobin, Hb A2-Hemoglobin A2, Hb A-Hemoglobin A, Hb F-Fetal hemoglobin, Hb S-Sickle Hemoglobin, RBC-Red blood cells, Ht-Hematocrit, MCV-Mean corpuscular volume, MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration.

Genotypic frequencies of different genotypes in the studied region were calculated. Results are given in Table 2. βS allele frequency was estimated at 1.6%, βThal at 0.9% and the normal allele βA at 97.5%. It was noticed that the observed frequency of SCA (0.73%), major β-thalassemia (0.36%) and the sickle/β-thalassemia (0.29%) was significantly higher than the expected number according to the HWE (0.03%, 0.01% and 0.03% respectively). Among Sickle cell trait, β-thalassemia trait subjects, the calculated genotypic frequency (3.21% and 1.71% respectively) was found significantly higher than the observed frequency (1.55% and 0.74% respectively).
Discussion

Hemoglobinopathies are the most common inherited disorders with a worldwide occurrence. Some geographical areas have high prevalence of these diseases, with nearly 80% of new hemoglobinopathies births are occurred in developing countries (4). In Morocco, these diseases represent one of the major public health problems. The exact data regarding the prevalence and spectrum of these diseases is still uncomplete. In the absence of an adequate strategy to manage hemoglobinopathies, its prevalence may continue to increase, and risk to pose economical and psychological burden on the affected individuals, their families and the whole society. Hence, the population needs to be screened for hemoglobin disorders so that appropriate measures for treatment and prevention can be taken.

This cross-sectional study is the first approach to understand the situation of hemoglobinopathies in the North of Morocco. A high rate was observed in rural (74.5%) compared to urban patients (25.5%). Compared to AA subjects distribution, hemoglobinopathies were statistically independent of the origin of the subjects (Odds ratio = 1.047, P-value > 0.05). In agreement with our results, Belala et al. reported that 81.13% of their studied patients in Kenitra province, Morocco were from rural areas (10). This may be a result of the rural character and the low genetic awareness in the region. In our study, the highest frequency was observed in Laouamra commune (45.6%) with the presence of diversity in the phenotypic expression and genetic aspects of hemoglobinopathies. This difference in distribution of the disease forms is often found in the small geographic areas from different countries (11). Our results are in agreement with those of Agouzal et al. and Laghmich et al. suggesting Laouamra as a hotspot of hemoglobinopathies in the country (6, 7). The concentration of patients in this area could be a result of three major risk factors: i) its proximity to Kenitra province; the most touched region reported in Morocco (12, 13). Migration flows within the two provinces contribute toward the increase of hemoglobinopathies range. ii) Its proximity to Chefchaouen province where the last autochthonous malaria case was registered in the country (14). iii) the high prevalence of consanguineous marriage in the northern Morocco population (15, 16). In fact, in inbred populations, the deleterious alleles are reintroduced into descendants, which promotes the incidence of these pathologies among Moroccan risked families (17, 18). The high rates of consanguineous marriages, migration and early mortality may explain the observed Hardy-Weinberg disequilibrium in our data set. These results reflect the urgent need for community awareness and mandatory screening programs in these high-risk areas. Our results estimated the allelic frequency of the βS gene at 1.6%. This is considered as high compared to the overall frequency reported in Morocco (1.2%) (19). Regarding the Mediterranean Basin; where hemoglobinopathies are widely spread; the average frequency of the βS gene is 1.89% and 0.8 to 3.5% in Tunisia and Algeria respectively (20, 21). The same prevalence was estimated at 0.39% in Spain and 0.57% in Portugal (22). The frequencies reach 30% in sub-Saharan African countries (21).
Of the 440 detected hemoglobin disorders carriers in our study, Sickle cell trait occurred at the highest frequency (42.3%), followed by β-thalassemia Trait (20.2%), sickle cell disease (19.8%), major β-thalassemia (9.8%) and sickle/β-thalassemia (8%). The same pattern is observed by Dahmani et al. showing that AS carriers were dominant in Kenitra province with a rate of 40.6%, 23% were SS patients, 3.2% were β-thalassemia trait and the double heterozygous S/β-thalassemia presents 2.9% out of all the patients (13). Our finding are in accordance with other studies including Saudi, Indian, Spanish, and Gabon populations (23–26). The high SCT prevalence in our data set could be the result of historical selective pressure of malaria in Larache province. Malaria provides survival advantage and hence, higher prevalence of hemoglobinopathies carriers (27). A study on the endemic history of malaria in these areas remains highly recommended.

Besides, 1.5 to 3% of the Moroccan population are carriers of β-thalassemia (28), which reveals that our allelic frequency (0.9%) is obviously underestimated. Regarding the Mediterranean countries, β-thalassemia rates reach 4.4% and 3% in Tunisia and Algeria respectively (29, 30). In Egypt, El-Beshlawy et al. reported that β-thalassemia carrier rates is varying from 5.3 to 9% (31). Many other studies indicate that β-thalassemia is common in the Gulf region such as Oman, UAE and Qatar with 5.3%, 2.4% and 17% respectively (32–34). Further studies including Iron quantification and other techniques are required for accurate determination of the prevalence of hereditary persistence of fetal hemoglobin (HPFH) and fusion chains such as delta-β-thalassemia.

Regarding patients’ gender, the sex ratio obtained was 1.01; the disease occurrence does not present a significant difference and it affects both sexes in a similar way ($\chi^2 = 0.125, P\text{-value} > 0.05$). Comparing normal and abnormal subjects in our study, there was no statistical significance regarding the sex of the subjects (Odds ratio = 0.719, P-value > 0.05). The autosomal recessive inheritance of these diseases explains the absence of a preponderance of one sex over another (25, 35). A slight female predominance was detected among Dahmani et al. and Agouzal et al. studies subjects (6, 13). Other studies reported a slightly higher percentage of males (10, 36). These differences would be reliable to the demographic data of each cohort.

In term of patients age, our population study was particularly young with 66.5% under 20 years. Dahmani et al. detected the same result; 53.3% of its population have less than 16 years (13). This young age is a result of the earliness of the clinical manifestations of the hemoglobinopathies that become apparent after the first 6–9 months of age. Before that age, high levels of Hb F inhibit the sickling and reduces the severity of these diseases (37). Additionally, the delay of diagnosis and treatment lead to various complications (4, 38–40), subsequently the death of those affected during the first few years of life with reported excess mortality reaching up to 92% (37). The limited medical service resources makes the access to optimum care more difficult for the majority of the patients. The main constraints are the high cost of management, the availability of safe blood for transfusions and iron chelating agents. In the absence of transfusion, children die before the age of six years and if transfused and non-chelated they usually die before 20 years (12).

**Conclusions**

Our study is the first one in Morocco to determinate the pattern of hemoglobinopathies in a Moroccan population. Our results show high frequencies of these abnormalities especially among young children in the studied region. It highlights hemoglobinopathies as severe public health in the studied province. Since no effective cures are available for these diseases, the major approaches to the control and management of these diseases are population screening, genetic counseling and prenatal diagnosis, and management of symptoms. The appropriate coverage may help to reduce the diseases prevalence and decrease both economic and psychological burden on patients.

**Abbreviations**

Hb  
Hemoglobin.

WHO  
World Health Organization.

Hb A2  
Hemoglobin A2.

Hb A  
Hemoglobin A.

Hb F  
Fetal hemoglobin.

Hb S  
Sickle Hemoglobin.

RBC  
Red blood cells.

Ht  
Hematocrit.

MCV
Mean corpuscular volume.
MCH
Mean corpuscular hemoglobin.
MCHC
Mean corpuscular hemoglobin concentration.

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Princess Lalla Meriem hospital ethics committee, following the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects and/or their guardians’ prior recruitment.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests

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**Authors' contributions**

AIFZ contributed for the study conception acquisition of data and analysis. AIFZ and LA performed the design and writing of the manuscript. GNN, BA, and BMM approved the analyzed literature data and contributed to the revision of the manuscript. All authors read and approved the final manuscript.

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**Figures**

**Figure 1**

Map of the study area.

**Figure 2**

Age distribution of the different hemoglobinopathies patients.
Figure 3

District wise distribution of hemoglobinopathies in Larache province.