

Screening of Hemoglobin Disorders in Referral Cases to the Hospital's Laboratory in Northeast Iran

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ABSTRACT

Determining the frequencies of hemoglobin disorders in referral cases to the hospital's laboratory for designing the best program for prevention, early diagnosis and treatment of these disorders. Hemoglobin electrophoresis and CBC were performed on 6033 cases referred to Imam Reza hospital, Mashhad, Iran from 2004 to 2009. Normal and abnormal electrophoretic patterns were identified in 77.55% (4679 cases) and 22.44% (1354 cases), respectively. The most common hemoglobin disorders, in order of frequency, were β -thalassemia minor (19.44%), Hb D (1.63%), Hb S (0.38%), thalassemia intermedia to major (0.24%), thalassemia major (0.23%), $\delta\beta^0$ -thalassemia (heterozygous) (0.18%), hereditary persistence of fetal hemoglobin (heterozygous) (0.1%), Hb H (0.1%), Hb C, E or O (0.07%), HbLepor (heterozygous) (0.03%), Hb D/ β^+ -thalassemia (0.02%) and Hb S/ β^+ -thalassemia (0.02%).

Based on our study hemoglobin disorders are a common problem in this region as the abnormal results in electrophoresis were observed in the 22.44% of individuals. These results show the importance of a premarital screening program for hemoglobin disorders in this geographic area.

Keywords: Hemoglobin disorders, Thalassemia, Hemoglobinopathy, Hemoglobin D, Hemoglobin S, Hemoglobin H

ÖZET

Kuzeydoğu İran da Hastane Laboratuvarına Gönderilen Hastalarda Hemoglobin Bozukluklarının Taranması

Bu çalışmanın amacı hastane laboratuvarına sevk edilen hastalarda hemoglobin bozukluklarının sıklığının belirlenerek, önleme, erken tanı, ve tedavi programlarının hazırlanmasında rehber oluşturmasıdır. İmam Rıza hastanesi, Meşhed, İran'da 2004-2009 yılları arasında, hemoglobin elektroforezi ve tam kan sayımının yapıldığı 6033 olgu değerlendirilmeye alındı. Normal ve anormal elektroforez modelleri sırasıyla %77.55 (4679 olgu) ve %22.44 (1.354 olgu), tespit edilmiştir. En yaygın hemoglobin bozuklukları, sıklık sırasına göre, β -talasemi minör (%19.44), Hb D (1.63%), Hb S (0.38%), majör talasemi intermedia-majör (%0.24), talasemi majör (%0.23), $\delta\beta^0$ -talasemi (heterozigot) (%0.18), kalıtsal kalıcı fetal hemoglobin (heterozigot) (%0.1), Hb H (%0.1), Hb C, E veya O (0.07%), HbLepor (heterozigot) (%0.03), Hb D/ β^+ -talasemi (0.02%) ve Hb S/ β^+ -talasemi (% 0.02). Çalışmamızda hemoglobin bozukluklarının bu bölgede önemli bir problem olduğu görülmüştür. Elektroforez sonuçlarına göre anormal ob- sonuçlar bireylerin 22.44% de izlenmiştir. Bu sonuçlar bu coğrafi alanda hemoglobin bozuklukları için evlilik öncesi tarama programının önemini göstermektedir.

Anahtar Kelimeler: Hemoglobin bozuklukları, Talasemi, Hemoglobinopati, hemoglobin D, Hemoglobin S, Hemoglobin H

INTRODUCTION

Hemoglobin disorders are a worldwide problem. They have spread during migration from high prevalence areas such as the Mediterranean, Africa and Asia to low prevalence areas such as Europe and America. Although control programs have limited affected newborns and increased survival of the patients, these disorders are still one of the most common genetic problems even in the developed countries.¹ There are two main types of hemoglobin disorders: thalassemias with defects in the synthesis of a hemoglobin chain and hemoglobinopathies with defects in its structure.² Thalassemia carrier rate in Iran, located in the middle of the so-called thalassemia belt, is high.³ Some hemoglobinopathies particularly Hemoglobin D, named hemoglobin D Iran, and hemoglobin S have also high frequency in this region.⁴ In the recent decades, due to administration of a preventing national program for thalassemia, including premarital screening, prenatal diagnosis and therapeutic abortions, the prevalence of thalassemias have decreased to some extent, but still these disorders are a serious health problem.³

Characterizing the frequencies of hemoglobin disorders in different geographic areas helps the health care providers to design the best program for prevention, early diagnosis and treatment of these disorders particularly appropriate for specific ethnic groups.

MATERIAL AND METHODS

This study financially was supported and ethically approved by the vice chancellor of research of Mashhad University of Medical Sciences, Iran. As screening for hemoglobin disorders, we performed hemoglobin electrophoresis (by Helena instrument, France) and complete blood count (CBC) (by Sysmex K-21, Japan) for all patients referred to our laboratory from pediatric, hematology and internal wards as well as the premarital genetic counseling clinic of Imam Reza teaching hospital (the biggest hospital in Mashhad, Khorasan province located in the Northeast of Iran) from 2004 to 2009. Subjects with a history of blood transfusion during 3 past months before admission were excluded from the study. In this period of time, 6033 samples were analyzed.

Procedure: After obtaining a short medical history, 2 milliliters blood was taken (using ethylenediaminetetraacetic acid (EDTA- K2) as anticoagulant) and CBC and hemoglobin electrophoresis on cellulose acetate at alkaline PH were performed within 24 hours of sampling. Regarding the fact, cellulose acetate electrophoresis doesn't separate hemoglobin (Hb) S, D, G and Lepor as well as Hb A2, C, E and O, so high performance liquid chromatography (HPLC) was used for separation of these hemoglobins.² We also perform sickling test for the confirmation of Hb S. Hb F was confirmed by alkaline denaturation test and borderline Hb A2 results (3.3%-3.7%) were rechecked by column chromatography. In case of suspicion to a Hb H band in electrophoresis, supra-vital staining of blood with brilliant cresyl blue was done for evaluation of Hemoglobin H inclusions.

In cases over 2 years old, Hb A > 97%, Hb F < 2-3% and Hb A2 < 3.5% were considered normal. β^0 -thalassemia major (β^0 -Thalassemia) was defined as a Hb F above 95% and Hb A2 for the rest (with no Hb A); thalassemia intermedia to major (β^+ -Thalassemia) was defined as anemia with 30-95% Hb F, and the presence of Hb A (Hb A2 may or may not be increased) and finally, β -thalassemia minor (Heterozygous state) was defined as Hb A2 > 3.5% , occasionally with slightly increased Hb F (1-3%) accompanied by characteristically elevated RBC, decreased MCV and MCH and usually normal MCHC (2, 5).

Heterozygous $\delta\beta^0$ -thalassemia was characterized as 5-20% Hb F, with or without decreased Hb A2 and the remainder Hb A with heterogenous pattern of HbF in acid elution test along with thalassemia phenotype in CBC. Heterozygous hereditary persistence of fetal hemoglobin (HPFH) was characterized as 15-30% Hb F with homogenous pattern of HbF in acid elution test, Hb A2 < 2.1% with no significant hematologic abnormalities in CBC. Heterozygous Hb Lepor was diagnosed as Hb Lepor of about 10%, low Hb A2 and high Hb F (2-3%).

Sickle cell disease (Hb SS) was determined as over 80% Hb S, 1-20% Hb F, 2-4.5% Hb A2 and no Hb A and sickle cell trait (Hb AS) as over 50% Hb A, 35-45% Hb S and up to 4.5% Hb A2. Hb D was also characterized with similar hemoglobin pattern. Hb S/ β^+ -Thalassemia was diagnosed by Hb S was

Table 1. Hemoglobin disorder frequencies (% and No) in referral cases to the hospital's laboratory

Thal syndroms	% (n)	Hemoglobinopathies	% (n)
β-Thal (heterozygous)	19.44 (1173)	Hb D disease	0.17 (10)
δβ ⁰ -thal (heterozygous)	0.18 (11)	Hb D trait	1.46 (88)
Hb Lepor (heterozygous)	0.03 (2)	Hb S disease	0.13 (8)
Thal major	0.23 (14)	Hb S trait	0.25 (15)
Thal intermedia to major	0.25 (15)	Hb S/β ⁺ -thal	0.02 (1)
HPFH (heterozygous)	0.1 (6)	Hb D/β ⁺ -thal	0.02 (1)
Hb H	0.1 (6)	Hb C,E or O trait	0.07 (4)

Thal: thalassemia, HPFH: hereditary persistence of fetal hemoglobin

over 50%, Hb F 1-20%, Hb A 15-30% and Hb A2 4-6% with mild hypochrom microcytic anemia. Similar criteria were considered for diagnosis of Hb D/β⁺-Thalassemia.²

Descriptive statistics were computed by SPSS software (version 11.5).

RESULTS

We evaluated 6033 individuals (including 42% males and 58% females) with age range of 1-88 years and mean of 26(±1.5) years. Normal electrophoretic patterns were observed in 77.55% (4679 cases) and abnormal patterns in 22.44% (1354 cases). The frequency of thalassemias (and related disorders) was 20.29% and hemoglobinopathies was 2.15%. The most common hemoglobin disorders, in order of frequencies, were β-thalassemia minor (19.44%), Hb D (1.63 %), Hb S (0.38%), thalassemia intermedia to major (0.24%) and thalassemia major (0.23%) (Table 1). Among the hemoglobin disorders, thalassemias and related disorders and hemoglobinopathies included 90.5% and 9.5% of cases, respectively. Among hemoglobinopathies (127 cases), the frequencies of disorders were as follow: Hb D (78%), Hb S (18.9%) and Hb C, E or O (3.1%).

DISCUSSION

The frequency of hemoglobin disorders varies noticeably with race and geographic area. The worldwide distribution of these disorders especially Hb S

are attributed to the distribution of malaria and possible resistance to malaria by heterozygous state. More common hemoglobin variants (Hb S, Hb C, Hb E, and Hb D) affect millions of people worldwide. Hb S is more common in African population; Overall prevalence of Hb S and Hb C trait in African Americans are about 8% and 3%, respectively. Probably, Hb E is the most common hemoglobinopathy worldwide and the third most common in the United States after Hb S and Hb C. Hb E is most common in Southeast Asia especially Thailand with a prevalence of 50% in some areas of this country.⁶ Hb D has some variants; The most common variant is Hb D Punjab (an area in India) with a prevalence of about 3% in this area and another variant is Hb D Iran.²

As shown in table 1, we found high frequencies of some hemoglobinopathies in our population that should be considered in the national screening program. We detected some differences in the frequencies of hemoglobinopathies compared with other reports so that the most common hemoglobinopathies in our study in order of frequencies were Hb D, Hb S and Hb C, E or O. We observed only rare cases (4 cases) of Hb C, E or O, but for some limitations we couldn't differentiated them. In a study by Ashtiani et al for determination of prevalence of hemoglobinopathies in the capital city of Iran (Tehran), similar results were obtained.⁴ in a study in Oman for evaluation of hemoglobin disorders on children under 5 years old, the following results were seen: sickle cell trait 6%, β-thalassaemia 2%, Hb D 0.6%, Hb E 0.3% and Hb C 0.02%.⁷ In this

study (on different group of population in comparison to our study) the most common hemoglobin disorder was sickle cell trait. Hb S/ β^+ -thalassemia is the third most common sickling disorder following Hb SS and Hb SC in African Americans but we detected only one such a case, probably due to a low frequency of Hb S disorders in our geographic area.

Thalassemias occur predominantly in Mediterranean region, Africa and Asia.² β -thalassemia genes especially are frequent in Greece and Sardinia and α -thalassemia genes in Southeast Asia.^{2,5} Approximately, 3% of world population carry β -thalassemia gene.⁵ In Iran, Thalassemia is more common in the north (Caspian Sea coast) and the south (Persian Gulf and Oman Sea coasts) areas. The overall prevalence is approximately 3 to 100 patients per 100,000 in different provinces.³ We also found high prevalence of β -thalassemia in our study. β -thalassemia major is the most common autosomal disorder in Iran⁸ and it is estimated that over 25000 cases of thalassemia major currently live in Iran.^{9,10} We also detected the significant numbers of thalassemia major and intermedia (table 1). In a study by Sachdev et al in India on 2600 cases for determination hemoglobin disorders, abnormal hemoglobins were seen in 327 cases (12.5%) including 15 cases of β -thalassemia major, 16 cases of β -thalassemia intermedia, 232 cases of β -thalassemia trait (8.9%) and 13 cases of Hb D Punjab and other disorders (Hb S, Hb E, Hb D Iran, Hb Q, Hb Lepore) for the rest (11). Similar to our results, predominant abnormalities were β -thalassemia trait and Hb D in Sachdev et al report.

In premarital screening programs in Saudi Arabia, Turkey and Iraq (neighbor countries of Iran) prevalence of β -thalassemia trait has been reported 3.4%, 2-2.8% and 3.7%, respectively.¹²⁻¹⁵ Although in these researches the most common hemoglobin disorders were β -thalassemia trait, but lower frequencies of β -thalassemia trait in these studies in comparison with our study probably originate from different case selection criteria (some patients with general complaints are also included in our study). Marouf et al study on 2,386 samples of hemoglobin electrophoresis in a hospital in Kuwait showed that the most commonly diagnosed hemoglobin disorders were beta-thalassemia minor (14%), sickle cell trait (6%), sickle cell anemia (0.9%), S/ β^0 -thalasse-

mia (0.8%) and S/ β^+ -thalassemia (0.8%).¹⁶ In this study, similar to ours, hemoglobin electrophoresis were abnormal in 23.5% and the most common disorder was β -thalassemia minor, but the most common hemoglobinopathy was Hb S and they identified only one case of Hb D Punjab. HPHF is found in about 0.1% of African Americans.² We only characterized a few cases of HPHF and $\delta\beta^0$ -thalassemia (heterozygous) in our research.

Hemoglobin H disease is more common in Southeast Asia, but it is also observed in Mediterranean and middle east area; and it is very rare in black people.^{2,5} We diagnosed rare cases of hemoglobin H in this study. Silent carrier and β -thalassemia trait don't show any abnormalities in hemoglobin electrophoresis. Therefore, we couldn't diagnose these disorders.

Based on the fact that consanguineous marriages are common in Iran and premarital screening is being carried out for thalassemia; we also suggest that at least in these kinds of marriages, premarital screening of hemoglobin variants especially Hb S and Hb D being considered in the preventive program. Respecting our limitations, we couldn't differentiate Hb C, E or O from each other. This was also true for Hb D variants, and diagnosis of β -thalassemia traits merely based on electrophoresis was impossible.

To conclude, hemoglobin disorders were a common problem in this population as abnormal results in electrophoresis were observed in 22.44% of individuals. The most common disorders were beta-thalassemia minor, thlassemia intermedia and major, Hb D, and Hb S. These results show the importance of the premarital screening program for hemoglobin disorders especially for thalassemias and some hemoglobinopathies.

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