Assessment of Data Regarding Thalassemias and Hemoglobin Variants From a Tertiary Referral Hospital Laboratory in Turkey

Bir Üniversite Hastanesinde Hemoglobinopati ve Talasemi Verilerinin Değerlendirilmesi

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ÖZET

Amaç: Hemoglobinopatiler, globin fonksiyonlarını bozan bir grup hastalıktır. HPLC, hemoglobinopati taramalarında tüm dünyada en sık kullanılan tekniktir. Çalışmada, Türkiye'de bir üçüncü basamak hastane laboratuvarında 3 yıllık retrospektif hemoglobinopati sıklığı değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Üniversite hastanemize başvuran 2461 hastanın 3 yıllık laboratuvar ve hastane kayıtları incelenmiştir. Hemoglobinopati taramasında Tosoh G8 HPLC cihazı kullanılmıştır

Bulgular: HbA2>%3,5 olan 668, %3,5<HbA2<%4 olan 19 sonuç saptanmıştır.19 sonuçtan 10'u beta-talasemi minör olarak kabul edilmiştir. HbA2 ≥%4 olan 649 sonuç vardır ve sonculara göre 602 hasta beta-talassemi minör olarak kabul edilmiştir. HbA2 düzeylerine göre, hastaların %25'i beta-talasemi minör olarak değerlendirilmiştir. HbF>%2 olan 391 hasta saptanmış ve HbA2<%3,5 olan 138 kayıt ayrıntılı incelemeye alınmıştır. Varyant analizi veri kümesi, 53 hasta içermektedir. HbA2≤%3,5 ve HbF≤%2 olan, varyant pikleri mevcut kayıtlar incelenmiş ve 28 vaka saptanmıştır. En sık görülen Hb varyantları sırasıyla HbH, HbS, HbD, HbC, HbE ve HbO-Arab'dır.

Sonuç: Çalışmada en sık varyantlar sırasıyla HbH, HbAS, HbAD, HbAC, HbAE ve HbO-Arab olarak bulunmuştur. Beta talasemi minör sıklığı ülkemizin gelen popülasyonundan yüksek olarak %25 bulunmuştur. Özellikle hematolojik malignensiler, herediter sferositoz ve demir eksikliği anemisi gibi eşlik eden durumlar hemoglobinopati değerlendirmesinde akılda tutulmalıdır.

Anahtar Kelimeler: Talasemi, Hemoglobinopati prevalansı, Üniversite hastanesi, Üçüncü basamak hastane

ABSTRACT

Objective: Hemoglobinopathies are an array of disorders that affect hemoglobin (Hb) function. HPLC is by far the most common technique used for detection of hemoglobinopathies worldwide. The aim of this study is to evaluate 3-year retrospective data of a tertiary hospital in Turkey according to prevalence of hemoglobinopathies.

Material and Methods: A three-year data of 2461 patients admitted to our university hospital laboratory were assessed by scanning laboratory and hospital records. Tosoh G8 HPLC instrument was used for screening.

Results: 668 results with HbA2>3.5% and 19 results with 3.5%< HbA2 <4% were detected. 10 of 19 patients were considered as beta-thalassemia. Number of patients with HbA2 ≥4% were 649 and 602 of them were assessed as beta-thalassemia-minor. 25% of patients were assessed as beta-thalassemia-minor, based on HbA2 levels. 391 patient results were found to be HbF>2% and 138 of them with HbA2<3.5% were further investigated. Variant analysis dataset contains 53 patients. Samples with HbA2≤3.5% and HbF≤2% showing variant peaks were scanned and 28 cases were detected. The most common variants were found to be HbH, HbS, HbD, HbC, HbE and HbO-Arab, respectively.

Conclusion: In this study, the most common variants were found to be HbH, HbAS, HbAD, HbAC, HbAE and HbO-Arab, respectively. Moreover, the frequency of beta-thalassemia minor was found as 25% which is higher than general population prevalence of our country. Accompanying diseases especially hematological malignancies, hereditary spherocytosis and IDA should be kept in mind during evaluating hemoglobinopathy analysis.

Key words: Thalassemia, Hemoglobinopathy prevalance, University hospital, Tertiary referral hospital

INTRODUCTION

Hemoglobinopathies are an array of disorders that affect hemoglobin (Hb) function. Hb disorders are broadly classified into three categories: Structural hemoglobinopathies, thalassemias and hemoglobin variants (1). Though most of the clinicians consider thalassemias as a subtype of structural hemoglobinopathy, the medical definition and causative factors differentiate them. Structural hemoglobinopathies are caused due to structural variation in the alpha or beta globin chain, which may not exhibit any phenotypic effect, and generally arise due to single nucleotide polymorphisms (SNP) or point mutations, while thalassemias arise from decreased alpha or beta globin chain synthesis. Delta/beta thalassemia and hereditary persistence of fetal Hb (HPFH) are clinically characterized by increased HbF even in adulthood. The alpha globin gene is present in chromosome 16, while the beta globin gene is present in chromosome 11 (2). As on today, around 1600 Hb variants have been documented and the majority do not show any clinical manifestation or are asymptomatic (3). These variants have also been documented to show prevalence only in certain regional or ethnic groups. Whatever the genetics of the Hb disorder, most cause symptoms of anemia with differing severity. It has been documented that close to 7% of the world's population are carriers for Hb disorders (4). The nomenclature of the Hb variants is generally done according to the family name of the first detected case or residence of the propositus; eg. Hb-Lepore and HbD-Punjab. The most common Hb variants detected and studied world over include HbS, HbE and HbC (5). Other less common and rare Hb variants include Hb-Barts which is an alpha thalassemia syndrome. This disorder is characterized by deficiency in synthesis of the alpha globin chain which leads to formation of Hb with four γ chains. However, due to gene switch post birth, the quantity is reduced due to paucity in γ chain synthesis (6). Hb-Lepore is another Hb variant which arises due to cross-over of the delta and beta globin genes. Although heterozygotes are generally asymptomatic, when present in the

homozygous state or in concomitance with beta thalassemia, it can lead to severe anemia (7). Accurate interpretation of hemoglobin analysis is virtually impossible, especially for thalassemias, without the accompanying hematological information obtained from a complete blood count (CBC). Structural hemoglobin mutations will have variable hematologic changes depending on the specific mutation. The key hematologic feature in diagnosing thalassemias is microcytic hypochromic anemia. A mean corpuscular volume (MCV) <80 fL is often applied as the cut-off for thalassemia suspicion in order to maximize sensitivity (8). Although the hypochromic microcytic anemia observed in thalassemias also occurs in iron deficiency anemia (IDA), IDA is commonly accompanied by a decreased RBC count and increased red cell distribution width (RDW), while hemoglobin abnormalities tend towards an increased RBC count and normal RDW (9). These parameters are integrated into the Mentzer index (MCV/RBC count). Diagnoses of IDA may be accompanied by a higher Mentzer index (generally greater than 13) whereas beta thalassemias will generally exhibit a lower index (10). In order to distinguish thalassemia major and thalassemia intermedia HbF, HbA2 and total hemoglobin levels can be utilized (11).

Technologies available for the screening of hemoglobinopathies range from conventional electrophoresis to more sophisticated approaches, ie, high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) (12). HPLC is by far the most popular technology used for detection of hemoglobinopathies worldwide. Most HPLC analyzers are calibrated to estimate HbA2, HbF and HbA along with variants such as HbC, HbS and HbD. In contrast to agarose gel electrophoresis, CE is based on the principle of liquid flow wherein Hb variants are separated by an electro-osmotic force under alkaline conditions (13). After presumptive diagnosis of hemoglobin disorders using biochemical methods is made, characterization of deletions or mutations may be necessary to confirm the clinical phenotype and/or guide genetic counseling. There are a variety of molecular techniques, most PCR-based, available for the identification of different hemoglobinopathy mutations and deletions (14).

The prevalence of beta-thalassemia carriers in Turkey was reported to be 2.1% in all over Turkey (15). The most common Hb variants in Turkey are HbS, HbD and HbE, respectively (16). The aim of this study is to evaluate 3-year retrospective data of a tertiary hospital in Turkey regarding prevalence of Hb variants.

MATERIAL AND METHODS

In our study, a three year (January 2013 – December 2015) data from 2461 patients (1032M, 1429F) admitted to our university hospital biochemistry laboratory were assessed by scanning laboratory (complete blood count, Hb variant analysis result, iron parameters, mentzer index) and hospital records of patients. Patients records lacking any of the above tests were excluded from data set. Tosoh HLC-723 G8 cation exchange HPLC instrument (Tosoh Bioscience, Japan) was used to screen thalassemias and Hb variants.

RESULTS

HPLC screened both abnormal cases of thalassemia and hemoglobinopathies. Before reporting any variant, patient demographics, i.e., age, gender, clinical status, transfusion history, region of origin, were taken into consideration. For most abnormalities, anemia, iron status and Mentzer indices were assessed by complete blood count and related biochemical tests. For reported cases of low HbA2, verification was done using a complete blood count to evaluate Hb concentration and other red blood cell indices to confirm iron deficiency. If the only elevated parameter was HbF, HPHF or delta/beta thalassemia were considered. Complete blood count results, Mentzer

indices, ferritin and/or serum iron, serum iron binding capacity values were scanned in order to detect prevalance of beta-thalassemia-minor.

First part of the analysis was evaluation of HbA2 levels. 668 samples (27.1% or 668/2461) with HbA2>3.5% and 19 samples (0.8%) with 3.5%< HbA2 <4% were detected. Tables 1 summarizes the distribution of patient results with elevated HbA2 levels and related conditions. 10 of 19 patients were considered as beta-thalassemia-minor according to their complete blood counts, Mentzer indices (<13), ferritin and/or serum iron, serum iron binding capacity values (normal). Increased levels of HbF (>2%) were along with this condition in 3 of these, whereas IDA was recognized in other 3 due to low ferritin levels. Two patients were considered as heterozygous HbS and HbD. Number of patients with HbA2 ≥4% were 649 (295 M, 359 F). 602 of them were assessed as beta-thalassemia-minor. 208 of them had also increased levels of HbF. 18 patients were considered as beta-thalassemia-intermedia, whereas only 1 patient was considered as a beta-thalassemia-major case. 10 patients were considered as heterozygous HbD and one patient was considered as heterozygous HbE. Presence of HbC along with beta-thalassemia was detected in 2 patients, whereas presence of HbD and beta-thalassemia was detected only in 1 patient. 15 results could not assessed due to insufficient data. To conclude, we can say that approximately total of 25% patients were assessed as beta-thalassemia-minor, based on HbA2 levels.

Second part of the analysis was to assess elevated HbF levels and variant peaks. 391 patient results were found to be HbF>2% and 138 of them with HbA2<3.5% were further investigated. Table 2 shows the distribution of patient results with elevated HbF levels and related conditions. 55 patients were beta-thalassemia-minor cases. 34 of them also had IDA. 18 cases were clinically normal and their HbF values were around 2.1 to 5.9%. 11 patients had hematological malignancies, whereas 10 patients are considered as beta-thalassemia-major. 11 patients had the diagnosis of hereditary spherocytosis. 8 patient were considered as alpha-thalassemia with HbA2 levels <1.7%, whereas 4 patients were considered as beta-thalassemia-intermedia. Two of them had also Down Syndrome and hypothroidism. Number of heterozygous HbD, HbS and HbE cases were only 1 for each. 1 patient had HbD and HbS variants. Another patient had HbS variant together with beta-thalassemia. 1 patient had also Behcet's Syndrome. 13 patients could not estimated due to insufficient data.

Variant analysis dataset contains 53 patients. Samples with HbA2≤3.5% and HbF≤2% showing variant peaks were scanned and 28 cases were detected. Tables 3 summarizes the data regarding variant hemoglobins. 7 patients were considered as alpha-thalassemia, whereas 11 patients had heterozygous HbS. 6 patients had heterozygous HbD. Also, 3 patients had heterozygous HbC. Only 1 patient had heterozygous HbO-Arab. The most common variants were found to be HbH, HbS, HbD, HbC, HbE and HbO-Arab, respectively.

Table 1. Elevated HbA2 levels and related conditions

Conditions	Number of patients (%)	Explanations
3.5%< HbA2 <4%	19 (0.8% of total)	10 (53%) of them considered as beta thalassemia minor- 3 of these patients had also IDA
HbA2 ≥4%	649 (26.3% of total)	602 (93%) of them considered as beta thalassemia minor

Table 2. Elevated HbF levels and related conditions

Relevant clinical condition	Number of patients
Clinically normal (delta/beta thalassemia and/or HPHF is suspected)	n=18 - HbF 2.1-5.9%
Thalassemia minor	n=21- HbF 2.1-8.9%
Thalassemia minor and IDA	n=34 - HbA2 2-3.1%
Hematological malignancies	n=11 - HbF 2.6-24.7%, HbA2 ↓/normal
Hereditary spherocytosis	n=11- HbF 6-11%
Thalassemia major	n=10- HbF>50%, (severe anemia, ferritin ↑)
HbH peak suspected	n=8 - HbA2 0.3-1.4%
Thalassemia intermedia	n=4- HbF %10-50, (mild anemia, ferritin ↓/normal)
Down syndrome and hypothroidism	n=2
HbAD	n=1
HbAS	n=1
HbAE /or Hb-Lepore peak suspence	n=1
HbSD	n=1
HbS and beta thalassemia	n=1
Behcet's syndrome	n=1
Insufficient data	n=13

Table 3. Presence of Hb variants and related conditions

Related clinical condition	Number of patients	Explanation
HbH peak suspected	15	n=14 - low HbA2; n=8 - high HbF
HbAS	15	n=4 - high HbF and/or HbA2 levels
HbAD	15	n=9 - high HbF and/or HbA2 levels
HbAC	5	n=2 - high HbA2
HbAE or Hb-Lepore suspected	2	HbA2 >10 %
HbO-Arab suspected	1	Normal HbA2 and HbF levels

CONCLUSIONS

In our study, we evaluated retrospective hemoglobin chain analysis data of a tertiary referral hospital regarding prevalence of hemoglobinopaties. According to our data, we could conclude that high HbF can found in clinically normal circumstances and it may be HPHF or delta/beta thalassemia according to previous data (2). In this study, the most common variants were found to be HbH, HbAS, HbAD, HbAC, HbAE and HbO-Arab, respectively. We also found 25% beta-thalassemia minor frequency which do not reflect the general population prevalence in our country (15,16). In another study, Güler and his colleagues found a ratio of beta thalassemia carrier of 2% and Hb S carrier ratio of 0.05% in the study conducted on couples whose carriers were identified in

premarital screening studies in Konya (17). As consanguineous marriages are still common in our country, the Hemoglobinopathy Control Program has been initiated to prevent the birth of new patients with premarital screening tests. 41 scanning centers, including Konya, where the carrier frequency is high in this frame, have been established (18). In both cases, our data are not compatible with this study, as our data include higher frequency rates. This may be due to the fact that selected cases with suspicious routine hematology test results are generally referred to our hospital for confirmation in Konya region.

It has been reported that the most common subtype of beta thalassemia carriership is the one which is presented with solely elevated levels of HbA2 which is compatible with our findings (19). Another study from Turkey reported that prevalence of HbS variant is the most common Hb variant in Turkey (0.3%) (20). HbS is the second most common variant according to our findings.

The limitation of this study is its retrospective design. Evaluation of data was only limited to electronic patient records and further analyses were not possible.

Our retrospective analysis of patients records showed that accompanying diseases especially hematological malignancies, hereditary spherocytosis and IDA should be kept in mind during evaluating hemoglobinopathy analysis. IDA should be corrected before the hemoglobin analysis is performed. Identification of carriers and pre-marriage genetic counseling activities can contribute greatly to community health and the economy of the country.

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