Analysis of thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey

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Turkey is located in a geographic area of the world where thalassemia syndromes and abnormal hemoglobins are common. In this study we aimed to evaluate the thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey. Among the patients admitted to our Pediatric Hematology or Hematology Clinic between January 1997-September 1999, hemoglobin electrophoresis of 3,228 cases investigated for anemia was done using high performance liquid chromatography. Beta thalassemia trait was diagnosed in 21.1%, beta thalassemia major in 0.2%, S-beta thalassemia in 0.37%, Hb D in 0.37%, Hb S trait in 0.32%, Hb E in 0.18%, Hb O-Arab in 0.12%, Hb G-Copenhagen in 0.09%, Hb D-Iran in 0.06%, Hb Lepore in 0.06%, Hb Hasharon in 0.03%. Our results demonstrate that people in the Aegean region of Turkey have a wide spectrum of thalassemia syndromes and abnormal hemoglobins.

Key words: abnormal hemoglobins, chromatography, hemoglobinopathy, thalassemia.

Inherited abnormalities of hemoglobin synthesis may be divided into two groups: the first group includes the thalassemia syndromes in which one or more of the normal polypeptide chains of hemoglobin are synthesized at a reduced rate, and the second group is the abnormal hemoglobin group, those characterized by structurally abnormal hemoglobin variants[1]. Most abnormal hemoglobins differ from normal hemoglobin in the substitution of a single amino acid for another. Recently, 698 abnormal hemoglobins have been recognized[1]. The prevalence of the thalassemia syndromes and abnormal hemoglobins varies considerably with geographic location and racial group. Thalassemia is considered the most common genetic disorder worldwide. Turkey is located in a geographic area of the world where thalassemia syndromes and abnormal hemoglobins are common[1-8].

Disorders of globin chain synthesis constitute a significant public health problem. Diagnosis may be required to confirm a provisional diagnosis such as sickle cell disease or beta-thalassemia major; to explain a hematological abnormality such as anemia or microcytosis; to identify an abnormality in the presymptomatic phase, as in neonatal screening; to predict serious disorders of globin chain synthesis in the fetus and offer the option of termination of pregnancy; to permit genetic counselling of prospective parents; and as preoperative screening for the presence of sickle cell hemoglobin[9].

Until recently the identification and quantification of hemoglobin variants required a sequence of test, each with inherent problems of reproducibility, accuracy, labor intensity and cost[10]. improved fully automated systems and reagents for techniques such as high performance liquid chromatography (HPLC) and isoelectric focusing have led to their introduction in many laboratories[10-21]. HPLC system is a rapid, simple and reliable method for diagnosis of hemoglobinopathies[15-21].

In this study, we aimed to evaluate the thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey, who were admitted to our Pediatric Hematology or Hematology Clinic and needed to be investigated for anemia, using HPLC.

Material and Methods

Among the patients who were admitted to our Pediatric Hematology or Hematology Clinic between January 1997-September 1999, hemoglobin electrophoresis of 3,228 cases who needed to be invstigated for anemia was done using HPLC. Anticoagulated (EDTA) blood samples of all patients were analyzed by the Bio-Rad Variant Hemoglobin Testing System (Bio-Rad Laboratories, California, USA) which utilizes the principles of cation-exhange HPLC. All of the patients' blood samples were first analyzed by the Bio-Rad Beta-thalassemia Short Program, and if unknown hemoglobin was determined, the Bio-Rad Variant Hemoglobinopathy Program was then used to evaluate the abnormal hemoglobin.

For the analysis, 5 µl of EDTA whole blood was automatically diluted with 1 ml of hemolyzing reagent. Hemolyzed specimens were loaded into a 100-place sampler compartment maintained at 12 ± 2 °C. Specimens were sequentially injected into analysis stream at a certain interval. Two dual-piston pumps and a preprogrammed gradient controlled the elution buffer mixture passing through the analytical cartridge. A dual-wavelength filter photometer detected the absorbance of Hb at the primary wavelength (415 nm) and corrected for nonspecific absorbance at the secondary wavelength (690 nm). Changes in absorbance were monitored and displayed as a chromatogram of absorbance versus time. Analysis data from the detector was processed by the built-in integrator and printed on the sample report.

The Variant Hemoglobinopathy Program uzitilized a retention time marker with assigned windows for hemoglobins F, A_0, A_2/E, D, S, and C. Retention times of hemoglobins contained in patient samples were normalized relative to the retention time of hemoglobin contained in the retention time marker. Analyte identification "windows" were intended to assist the laboratory in the interpretation of normal and abnormal hemoglobins detected in patient samples. The "windows" were established time ranges in which common hemoglobins have been observed to elute using the Variant Hemoglobinopathy Program. The retention time was the center of the window. Retention time was measured from the time of sample injection to the maximum point of each peak. Analytes that were detected outside of a retention time window were labeled as "unknown x", which represented an abnormal hemoglobin. Interpretation of unknowns was done by hematologists by correlating the results with a previously studied and detected Hb specimen (Hb x was determined by comparing retention times with a known Hb x specimen).

Results

Beta thalassemia trait was diagnosed in 683 cases (21.1%), beta thalassemia major in eight cases (0.2%), and Hb H in five cases (0.15%). Abnormal hemoglobin variants detected in this study are shown in Table I.

Table I. Abnormal Hemoglobin Variants Detected in Our Patients

Hemoglobin variant	n (%)*
Hb S+beta thalassemia	12 (0.37)
Hb S D	12 (0.37)
Hb S trait	10 (0.32)
Hb E	6 (0.18)
Hb O-Arab	4 (0.12)
Hb G-Copenhagen	3 (0.09)
Hb D-Iran	2 (0.06)
Hb Lepore	2 (0.06)
Hb Hasharon	1 (0.03)
Hb Montgomery	1 (0.03)
Hb Constant Spring	1 (0.03)
Hb Köln	1 (0.03)

* Percentage of all patients.

Discussion

Beta thalassemia is the most common thalassemia syndrome in Turkey. It also occurs at high frequencies among individuals of Mediterranean, East Indian, Middle Eastern, African or Southeast Asian descent[22,23]. It is almost evenly distributed over Turkey, and heterozygous frequency is reported to be around 2%[2,24,25]. The incidence of beta thalassemia is higher (about 10%) in some regions like western Thrace and the Mediterranean coast[5,26]. In our study the prevalence of beta thalassemia traits with increased HbA2 was 21.1%. We can not compare our results with the above mentioned studies, since our patient population consisted of patients admitted to our Pediatric Hematology or Hematology Clinic who needed to be investigated for anemia.

The prevalence and the distribution of alpha thalassemia are not well known in Turkey, since alpha thalassemia traits can be demonstrated chromatographically only in the newborn period,

and very time-consuming and expensive techniques are needed for diagnosis afterwards. We found Hb H in 0.15% of our patients. Cord blood studies from different regions of Turkey demonstrated Hb Bart's incidence as 1.6% and 3.6%[6].

The prevalence of abnormal hemoglobins was found to be 1.7% in our survey. Among abnormal hemoglobins, sickle hemoglobin is the most frequently encountered variant worldwide[27]; heterozygous sickle hemoglobin was present in 0.32% of our patients. In previous population screening tests, differing carriage rates of the gene were found, from 0.3% to 37%[7,24,28-31]. While the frequency of Hb S is lower in the Aegean region, it is higher especially on the southeast coast of Turkey, where an ethnic group called Eti-Turks lives[29-31]. We also detected Hb S+beta thalassemia in 0.37% of our patients. In geographic areas where thalassemia mutations and structural variants of alpha and beta globin genes are frequent (such as Southeast Asia and Africa), compound heterozygotes with a thalassemia mutation and a structural variant are common[22].

In previous reports, following the higher frequency of Hb S, Hb E was reported as the second most common abnormal hemoglobin in Turkey, with an incidence of 0.2% in Turks and 1.37% to 2.43% in Eti Turks[28,29,32,35]. Similar to our study, Arcasoy et al.[36] found Hb E in 0.11% of 3,600 patients with hematological findings. Instead of Hb E, Hb D was the second most common abnormal hemoglobin in our study, detected in 0.37% of the patients. Hb D is also a well known abnormal hemoglobin in our country, and can be found throughout Turkey[36,40]. Most of the abnormal hemoglobins reported previously were detected with starch eletrophoresis, and since Hb D moves like Hb S and Hb E moves like Hb O-Arab in starch electrophoresis, it is possible that some Hb S cases eported previously may be Hb D, and some Hb E cases may be Hb O-Arab[37]. But those abnormal hemoglobins can be separated more distinctly with HPLC[17,20,21]. A Hb E variant, Hb E-Saskatoon, has also been detected in Turkey by Prozorova-Zamani et al. and Gürgey et al.[35,41]. Mild hypochromic microcytic anemia is reported in heterozygous Hb E cases, as was present in our patients, whereas those hematologic features are normal in cases with Hb E-Saskatoon. But the HPLC program we used could not demonstrate Hb E-Saskatoon. For further differential diagnosis, mutation analysis such as DNA-sequencing may be necessary.

Hb O-Arab and Hb Lepore can be found with decreasing frequency in the Turkish population, and Hb Köln, Hb Hasharon, Hb Montgomery, Hb G-Copenhagen, and Hb Constant Spring detected in our study are among the rare abnormal hemoglobin variants. There are more than 25 rare hemoglobin variants reported in Turkish patients[40,42-49].

Since Turkey is located in a geographic area of the world where thalassemia syndromes and abnormal hemoglobins are common, and because our results also demonstrate that people in the Aegean region of Turkey have a wide spectrum of thalassemia syndromes and abnormal hemoglobins, it is important to identify an abnormality in the presymptomatic phase and give genetic counselling when necessary in our country.

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