The relationship between the type of mutation in the globin gene and the type and severity of sickle/beta-thalassemia disease in Jordanian patients

Zeyad J. El-Akawi, MD, PhD,
Hala S. Al-Remawi, MD, MRCP,
Khadijeh J. Al-Namarneh, MSc.

Thalassemias are inherited diseases caused by quantitative defects in the synthesis of one or more globin chains. Types are classified according to which globin chain synthesis is suppressed. The main 2 types are beta (β)-thalassemia and alpha (α)-thalassemia, which are caused by a defect in the synthesis of β- and α-globin chains of hemoglobin. Various forms of β-thalassemia have almost the same clinical manifestations that differ in severity.[1] Sickle cell disease includes a group of genetic disorders characterized by the presence of mutant hemoglobin called hemoglobin S, which is formed as a result of a point mutation in the second nucleotide of codon-6 of the β-globin gene. Hemoglobin S like hemoglobin A contains a tetramer of 2 α-chains, and 2 abnormal β-chains designated β^s chains. The clinical manifestations differ according to whether the patient has inherited 2 β^s-genes (sickle cell anemia) or one β^s-gene (sickle cell trait).[2] Sickle/β-thalassemia is a condition in which the patient has both mutations (double heterozygous) that result in HbS and β-thalassemia. The severity of this condition depends on the amount of normal hemoglobin (HbA) produced, which in turn depends on the thalassemia alleles, whether they are β^+ or β^0.[1,3,4] Accordingly, sickle/β-thalassemia is classified into; sickle/β^0-thalassemia, where there is no HbA production, and sickle/β^+-thalassemia in which HbA is produced in different concentrations. Patients whose cells produce large quantities of HbA will have a very mild form of the disease. In contrast, patients whose cells make few or no normal hemoglobin will have a disease as severe as the homozygous state of sickle cell anemia.[4] Sickle/β^+-thalassemia disease is mainly found in people of Mediterranean origin including Greece, Turkey, Italy, and the Middle East. It is also found in Yugoslavia (no longer exists), Africa, and North America. The type of β-thalassemia mutation affects the clinical severity of the disease. El-Hazmi et al,[5] in 1994 reported that the coexistence of HbS and β^+-thalassemia resulted in a milder course of the disease compared to HbSS. On the other hand, the presence of HbS and β^0-thalassemia showed variable clinical presentations including severe cases.[5] In a study performed on Sicilian sickle/β-thalassemic patients by Schiliro et al,[6] found that patients with a sickle/β^+-thalassemia have a milder clinical picture than those with sickle/β^0-thalassemia. Only 7.7% of patients with sickle/β^+-thalassemia presented with severe disease, while patients with sickle/β^0-thalassemia were varied, 22.6% were severe, 29.1% were moderate cases, and 47.3% had the mild form of the disease.[6]

In this study, we tested the molecular bases of β-thalassemia and the genotype, phenotype relationship in sickle/β-thalassemic patients in Jordan. The importance of this study is to better understand the molecular mechanisms that influence the severity of the disease, which might help in establishing prenatal diagnostic programs that will decrease the impact of this disease on the Jordanian population, and could be utilized in the diagnosis and evaluation of sickle/β-thalassemic cases.

Twenty-two sickle/β-thalassemic patients, 10 males and 12 females with age ranging between 3 and 23 years, attending both Princess Basma and Princess Rahma Teaching Hospitals in Irbid, Jordan were included in this study that was conducted from June 2003 to May 2004. The study was approved by the University Review Committee for Research on Humans and was performed at the Jordan University of Science and Technology. Informed consent was taken from patients or their parents. Blood samples were collected from patients before blood transfusion from the median vein in ethylenediamine-tetraacetic acid tubes. The blood samples were either used directly or stored in the refrigerator at 4°C until used (maximum storage time was 2 days). The deoxyribonucleic acid (DNA) was extracted from the blood samples using a commercial kit purchased from Promega, Madison, WI, USA according to the company instructions. Extracted DNA was amplified using the polymerase chain reaction (PCR) technique as follows: initial denaturation step at 94°C for 4 minutes, followed by 35 cycles each consisting of denaturation at 94°C for 10 seconds, annealing at 54°C for 15 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 3 minutes. The Taq DNA polymerase was obtained commercially from Promega, Madison, WI, USA, and the amplification mix was purchased from Vienna labs, Vienna, Austria. The DNA hybridization was performed following company instructions of the commercial kit. To determine the genotype of a sample we compared the staining pattern of the corresponding test strip with the available standards. Patients were classified into mild, moderate, and severe cases, by a specialist doctor, based on the information that had been obtained from their medical records.

Among the 20 types of mutations that occurred in the globin gene that were screened in this study for each patient, 6 different β-thalassemia mutations were detected. These mutations are IVS1-1, IVS1-110, IVS1-5, IVS2-1, IVS2-745, and codon 8/9. **Table 1** demonstrates the detected mutations with their β-thalassemia type and their frequency. As demonstrated, IVS1-110 and IVS2-1 mutations have the highest frequency, while IVS1-1 and codon 8/9 have the lowest. The following mutations were not detected in any of the patient's samples, -87 (C>G), -30 (T>A), codon 5, HbC, codon 6, codon 8, codon 22, codon 30, IVS1-116, IVS1-25, IVS1-2, codon 36/37, codon 39, codon 44, and IVS1-6. **Table 1** also demonstrates the match between the type of mutation detected and the type of β-thalassemia. Most patients with β^s/ IVS1-110 (89%) and β^s/ IVS2-1 (75%) genotypes have a severe clinical picture compared with other genotypes, where the clinical picture ranged from mild to moderate, and no severe cases demonstrated. The detection of 6 different types of β-thalassemia mutations along with HbS in this study, demonstrates the great heterogeneity of β-thalassemia alleles in Jordan. Four of these mutations (IVS1-110, IVS2-1, IVS1-1, IVS2-745) are Mediterranean in origin and they have been detected in almost all Mediterranean countries. Although the IVS1-5 mutation is Asian-Indian in origin, it has been detected in Jordan and some neighboring countries.[7] The IVS1-110 and IVS2-1 mutations account for 72.8% of all mutations. A mutation in codon 8/9, which was detected in only one patient, was the first to be reported in Jordan. The codon 8/9 mutation was first detected and characterized in an Indian patient.[8] It was also detected in one out of 174 β-thalassemia Greek patients.[9] The presence of this mutation in unrelated ethnic groups (Indian, Mediterranean) suggests that it might arise independently and it needs to be further investigated in other ethnic groups. Patients who have β^S/IVS1-110 and β^S/IVS2-1 genotypes should be given special attention and carefully monitored because most of these patients have a severe clinical picture.

In conclusion, our study showed that certain mutations (IVS1-110, IVS2-1) are associated with a severe phenotype, while others (IVS1-5, IVS2-745, IVS1-1, and codon8/9) are associated with a moderate or mild phenotype. Therefore, patients suspected to have sickle/β-thalassemia disease should be genetically evaluated as this might help in the treatment and the follow up of these patients.

Table 1 - Mutations with beta (β)-thalassemia type and its frequency.

Mutation type	Substitution	β-thalassemia type	Frequency (%)
IVS1-110	G>A	β^+	(36.4)
IVS2-1	G>A	β^0	(36.4)
IVS2-745	C>G	β^+	(4.5)
IVS1-5	G>C	β^+	(13.7)
IVS1-1	G>A	β^0	(4.5)
Codon8/9	+G	β^0	(4.5)

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From the Departments of Biochemistry and Molecular Biology (El-Akawi), Pediatrics (Al-Remawi), Faculty of Medicine, and Department of Medical Laboratory Sciences (Al-Namarneh), Faculty of Allied Health Sciences, Jordan University of Science and Technology, Irbid, Jordan. Address correspondence and reprint requests to: Associate Dean Zeyad J. El-Akawi, Department of Biochemistry and Molecular Biology, School of Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan. Tel. +962 (2) 7201000 Ext. 23783. Fax. +962 (2) 7201064. E-mail: zakawi@just.edu.jo

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