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HEMATOLOGIC FEATURES OF BETA-GLOBIN GENE MUTATION TYPE (βo) WITH HOMOZYGOUS BETA THALASSEMIA

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β-Tthalassemia is common genetic disorders in Turkey that characterized by the reduced synthesis (β^{+}) or absence (β^{o}) of the β-globin chains in the HbA molecule. In this study, we aimed to determine the effect of the mutation type of the β-globin gene on hematological values in homozygous β-thalassemia. This retrospective study was undertaken by Prenatal Diagnosis Centres of Cukurova University Medical Biochemistry at Adana. We evaluated 60 homozygous by implementing DNA sequencing analysis for mutations undetectable by conventional methods. 30 patients with β^{o} [FSC 44/ C-A] mutations and the other 30 patients with β^{o} [(IVS-II-1(G>A), CD39 (C>T), CD8 (-AA) CD39 C> T and CD36/37 (–T)] mutations, totally 60 patients were included in the study. Erythrocyte indices, HbF, HbA_2 levels were compared between the two groups. FSC 44/(-C) mutations were detected in patients. Hb, Hct, MCV in this group values were statistically lower than in patients with other detected mutations ($P < 0.05$). Between the two groups, there is no statistically different RBC, MCH, MCHC, HbF, HbA_2 levels ($P > 0.05$). For the first time in this study, it was found that the Hb, Hct and MCV value of the persons who carried the FSC 44/(-C) mutation were significantly lower than the persons who carrying other mutations. Between the two groups, there was no statistical difference in RBC, MCH, MCHC, HbF and HbA_2 levels. Awareness of FSC/44 mutation, which may have a heterogeneous clinical presentation, is required. We herein present the hematologic findings of a Turkish population carrying this mutation. This will also help to make a diagnosis.

K e y w o r d s: Homozygous β-thalassemia, FSC 44/(-C), erythrocyte indices, DNA sequence analysis.

Hemoglobinopathies are among the most common inherited diseases around the world. They are divided into two main groups: thalassemia syndromes and the structural hemoglobin variants [1, 2]. Thalassemia is a severe genetic blood disorder brought about by a mutation in the globin gene [3]. Beta thalassemia is a group of inherited autosomal recessive disease characterized by the presence of the defective synthesis chain β-globin part of the hemoglobin molecule [4]. They are characterized by the reduced synthesis (β^{+}) or absence (β^{o}) of the β-globin chains in the HbA molecule [5].

Three clinical and hematological conditions of increasing severity are recognized, the β-thalassemia carrier state, thalassemia major (homozygous) and thalassemia minor (heterozygous). The β-thalassemia carrier state, which results from heterozygosity for β-thalassemia, is defined by specific hematological features [5]. Individuals with beta thalassemia minor usually do not have any symptoms (asymptomatic) and people often are unaware that they have the condition [6]. Persons with thalassemia major usually come to medical attention within the first two years of life. These patients require lifelong RBC transfusions at regular intervals to survive [7].

The β-thalassemia is widespread throughout the Mediterranean Region, in Africa, the Middle East, the Indian subcontinent, and Burma, Southeast Asia and Indonesia [8]. It estimates that 4.5% of the worldwide population carries β-thalassemia mutants. The first β-thalassemia study for Turkey was published in 1985 [9].

On a molecular level, β-thalassemia mutations are quite heterogeneous, with more than 300 different mutations described in the literature. More than 200 different molecular defects are defined and 95% are caused by β-globin gene point mutations [10-12]. Heterogeneity of β-thalassemia is associated with more than 40 different mutations in Turkey [13]. So β-thalassemia is a major public health concern in Turkey; throughout the country, the gene frequency is expected to be 2.1%. But in certain regions, this figure increases to 10% [14]. Traditional hematological methods contributing to the identification of candidate carriers involve a primary screen based on a complete blood count (CBC), hemoglobin electrophoresis for Hb fractionation and initial quantification of HbA_2 and HbF levels [15]. The key components of the CBC include: Hb, red blood cell (RBC) number, mean corpuscular volume (MCV), and red cell distribution width (RDW) [16]. There is now much different polymerase chain reaction (PCR)-based techniques that can be used to diagnose the globin gene mutations. Direct mutation detection with Amplification Refractory Mutation System-PCR (ARMS-PCR) and Restriction Endonuclease Analysis of PCR fragments (PCR-RFLP) was performed by using amplified DNA from amniotic cells samples, while mutations in the parents were determined in advance [17]. DNA sequencing is one of the most widely used methods for analyzing DNA and has been successfully used to detect any mutation in the sequence being analyzed [18].

In this study, we aimed to determine the effect of the mutation type of the β-globin gene on the hematological values in homozygous β-thalassemia. We evaluated 60 patients by implementing DNA sequencing analysis for mutations undetectable by conventional methods.

Materials and Methods

The study was designed retrospectively among the β-thalassemia patients. A retrospective chart review was conducted for subjects seen at the Department of Biochemistry between 2008 and 2017. The study was performed in compliance with the national regulations on human experimentation and approved by the institutional committee.

Study participants. This retrospective study was conducted by Prenatal Diagnosis Centres of Cukurova University Medical Biochemistry at Adana. The medical files of 60 patients diagnosed with β-thalassemia were systematically reviewed in the study. DNA sequence analysis was performed for mutation scanning of the β-globin gene.

Design. Clinical data were provided by a review of medical records. The results of hematological values were provided by the patient’s registration system. We evaluated 60 patients by implementing DNA sequencing analysis for mutations undetectable by conventional methods. 30 patients with β^o [FSC 44/ C-A] mutation and the other 30 patients with β^o [(IVS-II-1(G>A), CD39 (C>T), CD8 (-AA) CD39 C> T and CD36/37 (–T)] mutations, totally 60 patients were included in the study. The common mutations were screened for first by restriction fragment length polymorphism (RFLP) and amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) for each patient. Then any remaining uncharacteristic samples were analyzed by DNA sequencing to identify thalassemia mutations. Erythrocyte indices, HbF, HbA_2 levels were compared between the two groups.

Statistical analysis. Data are presented as descriptive statistics including means. Data were expressed as a mean ± standard deviation for quantitative variables, with ANOVA tests. $P < 0.05$ was considered to be statistically significant.

Result and Dıscussıon

In this study were originally investigated using a two-step diagnostic strategy in which the common mutations were screened for first by restriction fragment length polymorphism (RFLP) and amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) for each patient. Then any remaining uncharacteristic samples were analyzed by DNA sequencing to identify thalassemia mutations. Subsequently, 30 patients with β^o [FSC 44/ C-A] mutation and the other 30 patients with β^o [(IVS-II-1(G>A), CD39 (C>T), CD8 (-AA) CD39 C> T and CD36/37 (–T)] mutations, totally 60 patients were included in the study. DNA mutations sequence analyses were detected in 30 patients. The hematological values are shown in Table. FSC 44/(-C) mutations were detected in patients. Hb, Hct, MCV values were statistically lower in this group than in patients with other detected mutations ($P < 0.05$). Between the two groups, there was no statistical difference in RBC, MCH, MCHC, HbF, HbA_2 levels ($P > 0.05$).

β-Thalassemia is extremely heterogeneous in terms of both of genotype and phenotype, depending on the nature of β-gene mutation and the extent of impairment in β-globin chain production. To date, more than 350 β-thalassemia mutations have been reported in the IthaGenes database, 40 of which have

Hematological values in β-thalassemia patients depending on mutation type in the HBB gene

Variable	β-thalassemia β° (FSC 44/(-C)) Mean (min-max) ± SD	β-thalassemia β° (IVS-II-1(G>A), CD39 (C>T), CD8 (-AA) CD39 C> T and CD36/37 (–T)). Mean (min-max) ± SD	*P* value
Hemoglobin (g/dl)	6.8 (6.1-9.7) 2.04	7.6 (7.1-10.6) 1.13	< 0.05 (0.029)
Red Blood Cells (mil/mm^3)	3.71 (3.04-4.71) 1.02	4.01 (3.43-5.08) 0.94	> 0.05 (0.88)
Hematocrit (%)	21.3 (20.3- 28.2) 2,34	24.2 (23.9-32.7) 1.24	< 0.05 (0.041)
Mean corpus volume (fl)	58.56 (52.8-65.3) 6.32	63.3 (62.4-66.8) 3.75	< 0.05 (0.024)
Mean cell hemoglobin (pg)	17.1 (16.6-22.1) 1.15	18.2 (17.9-26.1) 1.05	> 0.05 (0.67)
Mean corpuscular hemoglobin concentration (g/dl)	31.43 (29.4-33.8)	33.65 (26.9-36.1)	> 0.05 (0.89)
Hemoglobin F (%)	1.1 (0.6.1.7) 0.56	1.4 (0.8-4.6) 0.47	> 0.05 (0.92)
Hemoglobin A2 (%)	3.7 (3.5-4.1) 0.28	4.34 (3.6-4.7) 0.30	> 0.05 (0.79)

also been reported from Turkey [5, 19]. As a rule, heterozygous carriers of β-thalassemia (one affected allele), are asymptomatic, and only altered laboratory values (low, normal, or slightly subnormal hemoglobin levels, slightly low mean cellular hemoglobin, low mean cell volume, low β:α-globin chain ratio on biosynthesis. The β chains of patients with homozygous β-thalassemia have normal structures but are produced in reduced and sometimes undetectable amounts. As a result of this globin chain imbalance, the thalassemia's have varying degrees of ineffective erythropoiesis and hemolysis. Some biochemical tests (Hb, MCV, RBC, MCH, HbF and HbA_2) are useful for identifying carriers of the thalassemia. When biochemical tests are not exhaustive, it is necessary to study the molecular globin genes [18]. Several studies have been carried out since the 1980s to identify β-globin gene mutations and the rate of finding new mutations significantly increased after the invention of PCR technique that can be used to diagnose the globin gene mutations, including the amplification refractory mutation system (ARMS), denaturing gradient gel electrophoresis (DGGE) and gap-PCR. Today DNA sequencing is one of the most widely used methods for analyzing DNA and has been successfully used to detect any mutation in the sequence being analyzed [20-23].

In this study, we evaluated 60 patients by implementing DNA sequencing analysis of the mutations undetectable by conventional methods. We aimed to determine the effect of the mutation type (β°) in the β-globin gene on the hematological parameters in β-thalassemia patients. FSC 44/(-C) mutation results or has resulted from a single base deletion (C) at codon 44 of HBB gene and creates a β° allele [24]. FSC 44/(-C) mutations detected in patients. Hb, Hct, MCV values were statistically lower than, with other detected mutations ($P < 0.05$). Between the two groups, there was no statistical difference in RBC, MCH, MCHC, HbF, HbA_2 levels ($P > 0.05$).

For the first time in this study, it was found that the Hb, Hct, and MCV values of the persons who carried the FSC 44/(-C) mutation were significantly lower than the persons who carrying other mutations and between two groups there was no significant difference in RBC, MCH, MCHC, HbF, HbA_2 levels ($P > 0.05$). Awareness of FSC/44 mutation, which may have a heterogeneous clinical presentation, is required. Prevention of homozygous β-thalassemia, the clinically severe subtype, is possible with prenatal diagnosis and simply by detecting carriers. This will also help to make a diagnosis.

ГЕМАТОЛОГІЧНІ ПОКАЗНИКИ МУТАЦІЇ (βо) ГЕНА БЕТА-ГЛОБІНУ ЗА ГОМОЗИГОТНОЇ БЕТА-ТАЛАСЕМІЇ

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Бета-таласемія – поширене в Туреччині генетичне порушення, яке характеризується зниженням синтезу (β^+) або відсутністю (β^0) ланцюгів β-глобіну в молекулі HbA. У цій роботі визначали вплив типу мутації гена β-глобіну на гематологічні показники за гомозиготної бета-таласемії. Це ретроспективне дослідження проведено центром перинатальної діагностики Університету Чукурова в Адані. Проаналізовано 60 гомозигот із використанням ДНК-секвенування для визначення мутацій, які неможливо виявити звичайними методами. У дослідженні брали участь 60 пацієнтів: 30 із мутацією βº [FSC 44/CA] і 30 з мутаціями βº [(IVS-II-1 (G> A), CD39 (C> T), CD8 (-AA) CD39 C> T і CD36/37 (-T)]. Порівнювали показники еритроцитів і рівні HbF і HbA2 між цими двома групами. Виявлено мутацію FSC 44/(-C). Значення Hb, Hct, MCV у цій групі були статистично нижче порівняно з пацієнтами з іншими виявленими мутаціями (P < 0,05). Вірогідних відмінностей в рівнях RBC, MCH, MCHC, HbF, HbA_2 (P > 0,05) між цими двома групами виявлено не було. Вперше продемонстровано, що показники Hb, Hct і MCV у пацієнтів із мутацією FSC 44/(-C) були значно нижче, ніж у пацієнтів з іншими мутаціями. Статистичних відмінностей в рівнях RBC, MCH, MCHC, HbF і HbA_2 між цими двома групами не встановлено. Заявлено про необхідність вивчення мутації FSC/44, яка може мати різні клінічні прояви. Наведено дані гематологічних досліджень у жителів Туреччини, які є носіями цієї мутації. Одержані дані можуть бути використані за постановки діагнозу.

Ключові слова: гомозиготна бета-таласемія, FSC 44/(-C), показники еритроцитів, ДНК-секвенування.

ГЕМАТОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ МУТАЦИИ (βо) ГЕНА БЕТА-ГЛОБИНА ПРИ ГОМОЗИГОТНОЙ БЕТА-ТАЛАССЕМИИ

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Бета-талассемия – распространенное в Турции генетическое расстройство, характеризующееся снижением синтеза (β^+) или отсутствием (β^0) цепей β-глобина в молекуле HbA. В настоящей работе определяли влияние типа мутации гена β-глобина на гематологические показатели при гомозиготной бета-талассемии. Это ретроспективное исследование проведено центром перинатальной диагностики Университета Чукурова в Адане. Проанализировано 60 гомозигот с использованием ДНК-секвенирования для определения мутаций, которые невозможно обнаружить обычными методами. В исследовании принимали участие 60 пациентов: 30 с мутацией βº [FSC 44/CA] и 30 с мутациями βº [(IVS-II-1 (G> A), CD39 (C> T), CD8 (-AA) CD39 C> T и CD36/37 (-T)]. Сравнивали показатели эритроцитов и уровни HbF и HbA_2 в этих двух группах. Обнаружена мутация FSC 44/(-C). Значения Hb, Hct, MCV в этой группе были статистически ниже по сравнению с пациентами с другими обнаруженными мутациями (P < 0,05). Достоверных различий в уровнях RBC, MCH, MCHC, HbF, HbA_2 (P > 0,05) между этими двумя группами обнаружено не было. Впервые показано, что показатели Hb, Hct и MCV у пациентов с мутацией FSC 44/(-C) значительно ниже, чем у пациентов с другими мутациями. Статистические различия в уровнях RBC, MCH, MCHC, HbF и HbA_2 между этими двумя группами не установлены. Заявлено о необходимости изучения мутации FSC/44, которая может иметь различные клинические проявления. Приведены данные гематологических исследований жителей Турции, являющихся носителями этой мутации. Полученные данные могут быть использованы при постановке диагноза.

Ключевые слова: гомозиготная бета-талассемия, FSC 44/(-C), показатели эритроцитов, ДНК-секвенирование.

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