Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Biochemistry

The Effect on Hematological Values of Beta-Globin Gene Mutation Type (β^o) in Patients with Beta Thalassemia

Gülüzar ÖZBOLAT*, Abdullah TULİ
Cukurova University, Faculty of Medicine Department of Medical Biochemistry, 01330 Adana, Turkey

Original Research Article

***Corresponding author**
Gülüzar ÖZBOLAT

Article History
Received: 01.04.2018
Accepted: 10.04.2018
Published: 30.04.2018

DOI:
10.36347/sjams.2018.v06i04.066

Abstract: The beta thalassemia is common genetic disorders in Turkey that characterised 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 at beta-globin gene on the hematological values in beta-thalassemia individual. This retrospective study was undertaken by Prenatal Diagnosis Centres of Cukurova University Medical Biochemistry at Adana. We evaluated 80 heterozygous individual by implementing DNA sequencing analysis for the mutations undetectable by conventional methods. 40 individuals with βo [FSC 44/ C-A] mutation and the other 40 individuals with βo [(IVS-II-1(G>A), CD39 (C>T), Cd 8 (-AA) Cd 39 C> T and CD 36/37 (–T)] mutations, totally 40 individuals were included in the study. Erythrocyte indices, HbF, HbA_2 levels were compared between the two groups. FSC 44/(-C) mutations detected in individual HbA_2 and RBC values were statistically higher than, with other detected mutations ($p < 0.05$). Hct, MCV, MCH, MHCH values were significantly lower ($p < 0.05$). For the first time in this study, it was found that the HbA_2 and RBC values of the persons who carrying the FSC 44/(-C) mutation were significantly higher than the persons who carrying other mutations and the Hct, MCV, MCH, MHCH values were found to be significantly lower than the other mutations. This will also help to make a diagnosis.
Key words: Beta thalassemia, FSC 44/(-C), erythrocyte indices, DNA sequence analysis.

INTRODUCTION

Hemoglobinopathies are the most common group of autosomal recessively inherited monogenic disorders in Turkey [1,2]. They are characterized by deletions or mutations in the genes encoding the alpha (α) and beta (β) globin chains of the hemoglobin molecule and are broadly classified as thalassemia and sickle cell disorders [2]. The β-thalassemia is caused by over 300 mutations of the adult β-globin gene [3]. They are characterised by the reduced synthesis (β^+) or absence (β^o) of the β-globin chains in the HbA molecule [4].

Depending on severity of hematological and clinical conditions, β-thalassemia is classified into three types, namely, β-thalassemia major, β-thalassemia minor and β-thalassemia intermedia [5]. Individuals with thalassemia major usually come to medical attention within the first two years of life. They often require regular blood transfusions and lifelong, ongoing medical care. Individuals with beta thalassemia minor usually do not have any symptoms (asymptomatic) and individuals often are unaware that they have the condition [6]. B-Thalassemia minor is clinically asymptomatic but some subjects may have moderate anemia [7].

β-thalassemia is a worldwide condition with an overall carrier frequency of 2-25%, with cases mostly reported from the Mediterranean region, including Turkey, the Middle East, Central Asia, India, the Far East and Africa it is no longer limited to these geographical areas due to migration to different regions of the World [8,9]. However, in each population, a handful of ethnic group-specific alleles accounts for roughly 90-93% of the β-thal alleles [10]. The first β-thalassemia study for Turkey was published in 1985 [11].The heterogeneity of β-thalassemia is associated with more than 40 different mutations in Turkey [12]. So β-Thal is a major public health concern in Turkey; throughout the country the gene frequency is estimated to be 2.1%, but in certain regions, this figure increases to 10% [13]. The 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 [14].The key components of the CBC

include: Hb, red blood cell (RBC) number, mean corpuscular volume (MCV), and red cell distribution width (RDW) [15]. There are now many different polymerase chain reactions (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 [16]. DNA sequencing is one of the most widely used methods for analysing DNA and has been successfully used to detect any mutation in the sequence being analysed [17].

In this study, we aimed to determine the effect of the mutation type at beta-globin gene on the haematological parameters in beta-thalassemia individual. We evaluated 80 individual by implementing DNA sequencing analysis for the mutations undetectable by conventional methods.

MATERIALS AND METHODS

The study was designed retrospectively among the β-thal individual. A retrospective chart review was conducted for subjects seen at Department of Biochemistry between 2008 and 2017.

Study participants

This retrospective study was undertaken by Prenatal Diagnosis Centres of Cukurova University Medical Biochemistry at Adana. The medical files of 80 heterozygous individual 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 was obtained through a review of medical records. The results of hematological values were obtained through the individual registration system. We evaluated 80 heterozygous individual by implementing DNA sequencing analysis for the mutations undetectable by conventional methods. 40individuals with βo [FSC 44/ C-A] mutation and the other 40individuals with βo [(IVS-II-1(G>A), CD39 (C>T), Cd 8 (-AA) Cd 39 C> T and CD 36/37 (–T)] mutations, totaly 80 individuals 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 individual. Then any remaining uncharacterized samples were analysed 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 mean ± standard deviation for quantitative variables, with ANOVA tests. P value < 0.05 was considered to be statistically significant.

RESULTS

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 individual. Then any remaining uncharacterized samples were analysed by DNA sequencing to identify thalassemia mutations.

Subsequently, 40 individuals with βo [FSC 44/ C-A] mutation and the other 40 individuals with βo [(IVS-II-1(G>A), CD39 (C>T), Cd 8 (-AA) Cd 39 C> T and CD 36/37 (–T)] mutations, totaly 80 individuals were included in the study. DNA mutations sequence analysis was detected in 40 individual. The haematological values are shown in table 1. FSC 44/(-C) mutations detected in individual HbA_2 and RBC values were statistically higher than, with other detected mutations ($p < 0.05$). Hct, MCV, MCH, MHCH values were significantly lower ($p < 0.05$). Between two groups there is not statistically difference HbF, Hb levels ($p > 0.05$).

Table-1: Haematological values were in Thalassemia individual

Variable	β-thalassemia βº (FSC 44/(-C)) Mean(min-max) ± SD	β-thalassemia βº (IVS-II-1(G>A), CD39 (C>T), Cd 8 (-AA) Cd 39 C> T and CD 36/37 (–T)). Mean(min-max) ± SD	P value
Hemoglobin (g/dL)	10.72 (8,3-13,7) 2.64	10.64 (8.5-13.6) 1.06	> 0.05 (0.672)
Red Blood Cells (mil/mm^3)	5,21(4.20-6.70) 1.25	4.12(2.33-5.28) 0.54	< 0.05 (0.045)
Hematocrit (%)	34.61 (27,1- 42,8) 2,12	36.50 (29.9- 44.7) 1.05	< 0.05 (0.039)
Mean corpus volume (fL)	63,86 (56,8-71,3) 7,48	73,15 (62.4-82.5) 2.26	< 0.05 (0,013)
Mean cell hemoglobin (pg)	19,92 (17,6-24,1) 2.15	24.84 (19.9-32) 1.05	< 0.05 (0,022)
Mean corpuscular hemoglobin concentration (g/dL)	31,43 (29,4-33,8)	33,65 (26.9-36.1)	< 0.05 (0,033)
Hemoglobin F (%)	2,18(0,7-0,4,5) 1.06	2.66 (0.9-5.6) 0.42	> 0.05 (0.78)
Hemoglobin A_2 (%)	4.95 (3.9-6.3) 0.24	3.97 (2.6-4.5) 0.32	< 0.05 (0,047)

DISCUSSION

Thalassemia is a globin gene defect that results in a decreased rate of synthesis of one or more of the globin chains and a reduced rate of synthesis of the hemoglobin [18]. 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 [19]. To date, more than 350 β-thalassaemia mutations have been reported in the IthaGenes database, 40 of which have also been reported from Turkey [3, 12]. Two subtypes are defined by totally absent (β^0) or partially reduced (β^+) production of normal β chains, respectively [20]. Most of the beta-thalassemia mutations are caused by point mutations, small deletions or insertions within the coding regions and the exon-intron junctions. The types of the mutation are typically ethnic specific [21]. These non-deletional mutations, small insertions or deletions, single base substitutions of one to a few bases are located within the gene. They downregulate the β globin gene via from transcription to RNA processing and translation of β globin mRNA. Approximately half of the non-deletional mutations completely inactivate the β gene with no β globin production resulting in β^0 thalassemia [22].

Some biochemical tests (Hb, MCV, RBC, MCH, HbF and HbA2,) are useful for identifying carriers of the thalassemia. When biochemical tests are not exhaustive, it is necessary to study the molecular globin genes [23]. Several studies have been carried out since 1980s to identify beta globin gene mutations and the rate of finding new mutations significantly increased after 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 [24,25]. Today DNA sequencing is one of the most widely used methods for analysing DNA and has been successfully used to detect any mutation in the sequence being analysed [26].

In this study; we evaluated 80 heterozygous individual by implementing DNA sequencing analysis for the mutations undetectable by conventional methods. We aimed to determine the effect of the mutation type (β^0) at beta-globin gene on the hematological parameters in beta-thalassemia individual. FSC 44/(-C) mutation is resulted from a single base deletion (C) at codon 44 of HBB gene, and creates a β^0 allele (27). FSC 44/(-C) mutations detected in individual HbA_2 and RBC values were statistically higher than, with other detected mutations ($p < 0.05$). Hct, MCV, MCH, MHCH values were significantly lower ($p < 0.05$). Between two groups there is not statistically difference HbF, Hb levels ($p > 0.05$).

CONCLUSION

For the first time in this study, it was found that the HbA_2 and RBC values of the persons who carrying the FSC 44/(-C) mutation were significantly higher than the persons who carrying other mutations and the Hct, MCV, MCH, MHCH values were found to be significantly lower than the other mutations. This will also help to make a diagnosis.

REFERENCES

1. Elisabeth K. Hemoglobinopathies. Dtsch Arztebl Int 2011; 108(31–32): 532–40.
2. Özbolat G. Yılmaz N. Döğüş Y. Tuli A. The pregnancy variable in women with heterozygous beta thalassaemia. Ejpmr, 2018,5(4), 98-100.
3. Naouma PC. Hemoglobinopatias e talassemias. Sao Paulo, Sarvier 2004.

4. Finotti A, Breda L, Lederer CW, et al. Recent trends in the gene therapy of β-thalassemia. J Blood Med 2015; 19; 6:69-85.
5. Sanctis VD, Kattamis C, Canatan D, et al. β-Thalassemia Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint. Mediterr J Hematol Infect Dis 2017; 9(1).
6. Chaudhary S, Dhawan D, Bagali PG. Compound heterozygous β+ β0 mutation of HBB gene leading to β-thalassemia major in a Gujarati family-A case study. Mol Genet Metab Rep 2016; 7: 51–53.
7. Liaska A, Petrou P, CGeorgakopoulos CD, β-Thalassemia and ocular implications: a systematic review. BMC Ophthalmol 2016; 16: 102.
8. Galanello R. Orig R. Beta-thalassemia. Orphanet J Rare Dis. 2010; 5: 11.
9. Foong C , Ho JJ , Loh CK, et al. Hydroxyurea for reducing blood transfusion in non-transfusion dependent beta thalassaemias. **Syst Rev 2016; 18:10.**
10. Çürük MA, Yalın E Aksoy K. Prevention of hemoglobinopathies in Turkey. Thalassemia Reports 2013; 3: 1.
11. Abuzenadah AM, Hussein IM. Damanhouri GA, et al. Molecular basis of β-thalassemia in the western province of Saudi Arabia: identification of rare β-thalassemia mutations. Hemoglobin 2011; 35(4):346-57.
12. Cürük MA, Zeren F, Genç A, Ozavci-Aygün S, Kilinç Y, Aksoy K. Prenatal diagnosis of sickle cell anemia and beta-thalassemia in southern Turkey. Hemoglobin 2008; 32:525–30.
13. Fettah A, Bayram C, Yarali N, Beta-globin Gene Mutations in Turkish Children with Beta-Thalassemia: Results from a Single Center Study. Mediterr J Hematol Infect Dis 2013; 5(1).
14. Basak AN. The molecular pathology of beta-thalassemia in Turkey: The Boğaziçi University experience. Hemoglobin 2007; 31(2):233-41.
15. Traeger-Synodinos J. Harteveld CL. Advances in technologies for screening and diagnosis of hemoglobinopathies. Biomark Med 2014; 8(1):119-31.
16. Lafferty JD, Crowther MA, Ali MA, et al. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. Am J Clin Pathol 1996; 106:201-5.
17. R Talmaci, D Coriu, L Dan, et al. Prenatal molecular diagnosis of β-thalassemia: report on the first two cases in Romania. J Med Life 2008;15; 1(2): 138–147.
18. Didone A, Nardinelli L, Marchiani M. Comparative study of different methodologies to detect the JAK2 V617F mutation in chronic BCR-ABL1 negative myeloproliferative neoplasms. Pract Lab Med 2016; 1; 4: 30–37.
19. Elhalfawy1 KH. Daıf A. Shaalan O. Detectıon of Common Beta Thalassemıa Mutatıons Among Egyptıan Patıents. Egypt. J. Genet. Cytol 2017; 46: 111-119.
20. Kamilia B. Mouloud Y. Moez G, et al. Effects of codon 39 (c>t) mutation on the changes of hematological parameters in children with beta-thalassemia major in the region of Batna, Algeria. Curr Pediatr Res 2016; 20 (2): 203-208.
21. Zhang J. He J. Mao X. Haematological and electrophoretic characterisation of β-thalassaemia in Yunnan province of Southwestern China. Genetics and Genomics Research 2017; 1:2.
22. Boonyawat B, Monsereenusorn C. Traivaree C. Molecular analysis of beta-globin gene mutations among Thai beta-thalassemia children: results from a single center study. Appl Clin Genet. 2014; 7: 253-258.
23. Thein SL. Molecular basis of β thalassemia and potential therapeutic targets. Blood Cells, Molecules and Diseases 2017; 17: 1079-9796.
24. Dell'Edera D. Benedetto M. Leo M. Identification of patients with defects in the globin genes by analysing blood parameters and genetic study: Report of five cases. Journal of Hematological Malignancies 2013;3:2.
25. Mahdavi MR, Karami H, Akbari MT, et al.Detection of Rare Beta Globin Gene Mutation [+22 5UTR(G>A)] in an Infant, Despite Prenatal Screening. Case Rep Hematol 2013.
26. Old JM. Antenatal Diagnosis of Hemoglobinopathies.Pediatric Hematology 1991; 91: 33-62.
27. Didone A, Nardinelli L, Marchiani M. Comparative study of different methodologies to detect the JAK2 V617F mutation in chronic BCR-ABL1 negative myeloproliferative neoplasms. Pract Lab Med 2016; 1; 4: 30–37.
28. Bazi MS, Moghaddam EM. Spectrum of β-thalassemia Mutations in Iran, an Update. Iran J Ped Hematol Oncol 2016; 6:3, 190-202..