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HEMOGLOBINOPATHIES PATIENTS AS GOLD STANDARD DNA SEQUENCING IN TURKEY

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ABSTRACT

Hemoglobinopathies constitute the most common monogenic disorders in Turkey, caused by mutations in the globin genes which synthesized the globin chains of hemoglobin. They are classified into two groups as structural hemoglobin variants and thalassemia. We want to showed that DNA sequencing method approach is considered the''gold standard' for DNA sequence analysis. The study was designed retrospectively among the 100 patients that prediagnosed Hemoglobinopathy undetectable the mutations with ARMS and RFLP. DNA sequencing was performed. In addition, erythrocyte the indice, HbF, HbA_2 levels were compared. Statistically; Man Witney and ANOVA test was performed. 100 samples with methods mentioned above unidentified mutation were examined by DNA sequence analysis. While the study can not be detected mutations in 39 individuals, it detected mutations in 41 individuals. Mutations detected in individuals MCV, MCH values were statistically lower than those of who can not be detected mutations ($p < 0.005$). HbA_2 and RDW values were significantly higher ($p < 0.05$). RBC, Hb, Hct, MCHC, significant differences were not found in HbF values. DNA sequence analysis is the one of most effective way unidentified mutations by other methods. Although the traditional methods as ARMS and RFLP are are time consuming and prolong the turnaround time for diagnosis. We think to be simpler and more quicker than ARMS and RFLP, made was it a good choice for rapid prenatal diagnosis of thalassemia and sickle cell disease in Turkey.

KEYWORDS: Hemoglobinopathies, DNA sequencing, mutations, ARMS, RFLP.

Hemoglobinopathies are the most common group of autosomal recessively inherited monogenic disorders in Turkey.[1] They are characterized by mutations or deletions in the genes encoding the alpha and beta globin chains of the human hemoglobin molecule and are broadly classified as sickle cell disorders and thalassemia.[2] Although hemoglobinopathies and thalassemia's are two genetically distinct disease groups, the clinical manifestations of both include anemia of variable severity and variable pathophysiology. The thalassemias are characterized by a genetic deficiency in the synthesis of beta-globin chains.[3] Thalassaemia syndromes are sub-classified based on the gene involved. They are characterised by the reduced synthesis (β+) or absence (βo) of the β-globin chains in the HbA molecule.[4] The clinical and hematological spectrum of beta-thalassemia ranges from silent carrier to clinically manifested conditions including severe transfusion dependent beta-thalassemia major and beta-thalassemia intermedia.[5,6] The molecular basis of thalassemia has been studied worldwide. More than 300 different beta-globin gene mutations have been characterized. 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.[7]

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 Hb A_2 and Hb F levels.[8] The key components of the CBC include: Hb, red blood cell (RBC) number, mean corpuscular volume (MCV), and red cell distribution width (RDW).[9] There are now many different polymerase chain reaction (PCR)-based techniques that can be used to diagnose the globin gene mutations. Molecular testing using direct mutation detection with Amplificaton Refractory Mutation System-PCR and Restriction endonuclease Analysis of PCR fragments was performed by using amplified DNA from amniotic cells samples, while mutations in the parents were determined in advance.[10] DNA analysis is used for definitive diagnosis of possible carriers, especially useful when the hematological and biochemical results are ambiguous.[11] 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. The DNA

sequencing approach is often considered the''gold standard' for DNA sequence analysis; however.[12]

In this study; We want to showed that DNA sequencing method approach is considered the''gold standard' for DNA sequence analysis. The individuals that come with hemoglobinopathy prediagnosis to us and was not determined the mutations with ARMS and RFLP methods. We evaulated 100 individuals that prediagnosed Hemoglobinopathy undetectable the mutations with ARMS and RFLP. ARMS and RFLP method unidentified individuals with mutations, DNA sequencing was performed. Individuals were evaluated retrospectively. In addition, erythrocyte the indices, HbF, HbA_2 levels were compared.

MATERIALS AND METHODS

Methods

The study was designed retrospectively among the 100 individuals that prediagnosed Hemoglobinopathy. A retrospective chart review was conducted for subjects seen at Department of Biochemistry between 2008 and 2016.

Study participants

This was a retrospective study design, based on review of records of patients seen at the Medical Sciences. Patient inclusion criteria were having a diagnosis of SCD by hemoglobin electrophoresis. A total of 100 patients hematological data were obtained through a review of medical records from the Department of Biochemistry with the confidentiality of information being preserved. We evaulated 100 individuals that prediagnosed Hemoglobinopathy undetectable the mutations with ARMS and RFLP. ARMS and RFLP method unidentified individuals with mutations, DNA sequencing was performed.

Design

Clinical data was obtained through a review of medical records. 100 samples with methods mentioned above unidentified mutation were examined by DNA sequence analysis. In addition, erythrocyte the indices, HbF, HbA_2 levels were compared and later compiled for statistical analysis.

Statistical analysis

Data are presented as descriptive statistics including means. The Man Witney and ANOVA test was performed.

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 patients. Then any remaining uncharacterized samples were analyzed by DNA sequencing to identify the rare and novel thalassemia mutations. This strategy was very successful and revealed a total of 41 Thalassemia mutations.

The haematological values are shown in table1. 100 samples with methods mentioned above unidentified mutation were examined by DNA sequence analysis. While the study can not be detected mutations in 39 individuals, it detected mutations in 41 individuals. Mutations detected in individuals MCV, MCH values were statistically lower than those of who can not be detected mutations ($p < 0.005$). HbA_2 and RDW values were significantly higher ($p < 0.05$). RBC, Hb, Hct, MCHC, significant differences were not found in HbF values.

Table 1. Haematological values.

Variable	Hemoglobinopathies Mean(min-max)	P
Hemoglobin (g/dL)	10.17 (6-14.08)	> 0.05
Red Blood Cells (mil/mm^3)	4.21(3.98-5.75)	> 0.05
Hematocrit (%)	34.97 (30,13- 43)	> 0.05
Mean corpus volume (fL)	81.03 (91,5-64,2)	<0.005
Mean cell hemoglobin (pg)	27.99 (36.5-18.9)	> 0.05
Mean corpuscular hemoglobin concentration (g/dL)	30,69 (33.59-27.5)	<0.005
Hemoglobin F (%)	2.14(2.6-0.5)	> 0.05
Hemoglobin A_2 (%)	4.38 (6.9-2.7)	< 0.05
Red Cell Distribution Width (RDW)	20.8(12.8-37.5)	< 0.05

DISCUSSION

Hemoglobinopathies constitute the most common monogenic disorders worldwide, caused by mutations in the globin genes that synthesize the globin chains of haemoglobin.[8] The hemoglobinopathies refer to a diverse group of disorders caused by a disruption of this normal pattern of globin gene expression. The disorders are characterized by either a reduced synthesis of one or more of the globin chains or the synthesis of a structurally abnormal Hb variant.[13]

There are now many different polymerase chain reaction (PCR)-based techniques 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. Each method has its advantages and disadvantages.[14] The DNA

sequencing should now routinely used to look for mutations in the Hemoglobinopathy. Generally sequencing is indicated if mutations are not detectable with the preliminary screening approaches described. Increasingly though, direct DNA sequencing is being used in difficult cases, for example, the finding of normal HbA_2 β thalassaemia is best dealt with by sequencing the β globin gene because there are a number of mutations known to be associated with this phenotype. Occasionally, normal HbA_2 β thalassaemia is found in association with the conventional β thalassaemia causing mutations. Thus in many cases of carrier screening, an accurate diagnosis requires expertise in the interpretation of the haematological results and confirmation of the genotypes by DNA analysis.[4,15]

At present, numerous methods including the restriction fragment length polymorphism (RFLP), amplification refractory mutation system (ARMS), have been used in previous studies on Hemoglobinopathies. However, these methods have been impractical for rapid and large-scale screening due to the high cost or requirement of specialized equipment and highly trained personnel. Also these methods are time consuming and prolong the turnaround time for diagnosis. DNA sequence analysis is the one of most effective way unidentified mutations by other methods. We think to be simpler and more quicker than ARMS and RFLP, made was it a good choice for rapid prenatal diagnosis of thalassemia and sickle cell disease in Turkey.

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