Kermanshah University of Medical Sciences

Journal of Kermanshah University of Medical Science

Journal homepage: Htpp://journals.kums.ac.ir/ojs/index.php/jkums

Original Article

The molecular analysis of mutations in exons 4, 11 and 21 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in cystic fibrosis patients in Kermanshah, Iran

Nasibe Karimi[1], Ali Bidmeshkipour[2], Keyghobad Ghadiri[3], Reza Alibakhshi[4]*

1. Dept. of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Iran.
2. Dept. of Biology, Faculty of Science, Razi University, Kermanshah, Iran.
3. Nosocomial Infectious Disease Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.
4. Dept. of Biochemistry, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Article Info

Keywords: CF, Kermanshah, 4029>G, D110H, CFTR

***Corresponding Author**:
Department of Biochemistry, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran,
Tel: +988337213330, +989181334404
Fax: +988337213330,

Email: ralibakhshi@kums.ac.ir; ralibakhshi@yahoo.com

Received: 21 August, 2016
Accepted: 15 January, 2017

J Kermanshah Univ Med Sci. 2017; 20(4): 147-51

Abstract

Introduction: Cystic fibrosis (CF) is a common genetic disorder in white populations with an autosomal recessive pattern, caused by mutations in the CFTR gene. The frequency of more than 1950 various mutations reported in the CFTR gene significantly varies in different populations. ΔF508 is a common mutation in exon 10, which is first addressed in the molecular analysis of the disease. Other exons are required to be investigated owing to failing to identify mutations in the patients. The present study was conducted to investigate mutations in exons 4, 11 and 21 of the CFTR gene using the sequencing method in CF patients in Kermanshah province, Iran.

Methods: The present descriptive study was conducted on all patients with CF presenting to the medical genetics center in Kermanshah in 2010-2011. After taking blood samples and extracting DNA using saturated NaCl solution, sequences of exons were amplified using PCR and sequenced for identifying mutations.

Results: The frequency of mutations was found to be respectively 0, 0 and 5.5% in exon 11, 21 and 4. The D110H mutation was found to be homozygous in one subject and heterozygous in another. Moreover, the 4029A>G polymorphism (12.9%) was found to be homozygous in two subjects and heterozygous in three others.

Conclusion: The D110H mutation is recommended to be included in the screening programs of the study population. The results obtained support the effects of ethnic and geographical factors on the distribution of CF mutations.

Introduction

CF; MIM# 219700 is caused by mutations in the CFTR gene at the 7q31.2 chromosomal location. This gene consists of 27 exons and encodes a 1480-amino acid glycosylated protein, which affects the flow of ions and functions as a chloride channel in epithelial membrane of the respiratory tract, the pancreas, the intestine, the male genital tract, the liver and sweat glands. The channel epithelial dysfunction is the main complication and the cause of clinical symptoms of CF, including chronic pulmonary disease, exocrine pancreatic insufficiency, elevated sweat chloride concentration (more than 60 ml/l) and male infertility [1-3]. CF prevalence is 1 in 2,500 newborn infants, with a carrier frequency of 1 in 25. More than 1950 CFTR mutations have been identified, with variable frequencies depending on the geographic and ethnic background. The highest prevalence is associated with exon 10 mutations, including the most common three-nucleotide deletion, ΔF508, with a frequency of 87% in Northern Europe and 28% in the Middle East. The frequency of the other mutations such as W1282X, G551D, N1303K and G542X is at least 1%. Moreover, there are 17 mutations with frequencies of 0.1%–0.9% in specific populations. Other mutations of the CFTR gene are rare and the abnormal prevalence of some mutations in special populations is probably caused by the founder effect [4]. The highest mutation in the CFTR gene occurs in the second nucleotide binding, which is encoded by exons 10 and 11. G542X, G551D, R553X and R560T are the common mutations reported in exon 11 and T/G1764 and A/T1773 the common polymorphisms. Exon 21 encodes part of the second nucleotide-binding domain in this protein. N1303K, N1303H, 4040delA, Q1313X and W1316 are the most common mutations in exon 21 and 4040A/G, 4050C/T, 4095+42T/C and 4096-283T/c the most common polymorphisms [5]. The factors affecting the diversity of the distribution and frequency of mutations in the CFTR gene in different populations include the selective advantage of heterozygotes, the founder effect and genetic drift. Therefore, conducting a molecular analysis is theoretically and practically crucial for determining the frequency and distribution of CF gene mutations in a region. Despite the lack of accurate epidemiological

information about CF, previous studies suggest the prevalence of CF in Iran [5]. Numerous clinical-oriented studies conducted across the world reported a wide spectrum of type, frequency and distribution for the CFTR gene mutations in different ethnic groups and populations. Research also suggests different mutation patterns in Iran compared to in its neighboring countries. Given the high rate of consanguineous marriage in Iran, the consequent possibility of disease transmission to next generations and the fatal nature of the disease, the frequency and pattern of mutations are required to be determined in different populations and regions across the country and pave the way for prenatal diagnosis and heterozygote identification. In addition, no comprehensive genetic studies have so far been conducted on patients in Kermanshah province, as the largest Kurdish region in the west of Iran with a population of 1,945,227. The present study was therefore conducted to investigate the CFTR gene mutations in CF patients using accurate sequencing techniques. The study conducted on the present study patients failed to determine the mutation in exon 10 in all the patients [6]. The present study thus addressed exons 11, 21 and 4 to identify mutations in the remaining patients.

Materials and Methods

The present study was conducted on 40 patients, including 18 males and 22 females at the age of 2 months-19 years, who presented over the period of February 2010 to August 2011 to the Medical Genetics Laboratory and the Center for Prenatal Diagnosis of Genetic Disorders in Kermanshah University of Medical Sciences, Kermanshah, Iran. Clinical symptoms, including acute or mild respiratory disorders, meconium ileum, growth disorders and abnormal stools, having a sibling with a history of the disease and positive sweat tests were used to diagnose the disease. The study patients were closely examined and some of them received pancreatic supplements or treatments for their respiratory problems. The inclusion criteria comprised having two positive sweat tests and those with borderline sweat tests were excluded. Mutations were thus investigated in 27 patients including 13 males and 14 females with two positive sweat tests and three consecutive generations living in Kermanshah province. The patients' clinical and demographic information was recorded in the questionnaire after obtaining informed consent from their parents. In order to conduct molecular tests, 8 ml of blood sample was collected from each subject in tubes containing EDTA. DNA was extracted from the samples using saturated NaCl solution and the DNA concentration was determined using NanoDrop spectrophotometers.

Genotype determination

Exons 11, 21 and 4 were amplified in the ABI thermocycler using the primers studied by Alibakhshi et al [13]. Table 1 presents the concentration of the materials used to amplify each exon in a volume of 50 µl.

Table 1. The concentration of the materials used for exon amplification in PCR

Material	Concentration
Taq.Polymerase 2 units	1.5 mM
DNA Template50 ng	
each primer 25 pmol	
dNTP 200 uM	
10x PCR Buffe2.5 ul	
MgCl2	

The thermal cycle used to amplify all the three exons were as follows: 5 min at 94 ˚C, 30 sec at 95 ˚C (29 cycles), 30 sec at 58 ˚C, 1 min at 72 ˚C followed by 7 min at 72 ˚C. After completing the PCR and observing specific bands on the agarose gel, the product was prepared for sequencing. The QIAquick PCR Purification kit was first used for purification and the purified products were amplified using the BigDye Terminator v3.1 Cycle Sequencing kit (ABI Co., US). The PCR products were then placed in the ABI 3130 sequencer using ethanol precipitation and 15 µl of formamide. The sequences were compared with the wild-type CFTR nucleotide sequence using Seqscape software (Applied Biosystems).

Findings

Patient Phenotype

A total of 54 chromosomes were investigated in 27 nonrelative patients to identify mutations in exons 11, 4 and 21 of the CFTR gene. Males comprised 48.15% (13) of the subjects and females 51.85% (14). The subjects' age was 2 months-19 years and their mean age was around 5 years. Moreover, 66% (18) of the patients were found to have consanguineous parents with a maximum degree of relationship of 3. All the patients were also found to suffer from mild to acute respiratory problems, while 80% developed gastrointestinal problems such as weight loss and steatorrhea.

Genotype Determination

Disease-causing mutations were observed only in exon 4 of the CFTR gene in two of the patients from the cities of Kermanshah and Saqez (table 2), including one homozygous and one heterozygous for the D110H mutation (5.5%) (figure 2). Two patients were found to be homozygous and 3 heterozygous (12.9%) for the A/G4029 polymorphism in exon 21 (figure 1).

Table 2. Frequencies of CFTR mutations identified in the study patients

Exon/ Intron	Mutation nucleotide change	Mutation legacy name	No. of Patients		Total alleles
			Homo	Hetero	
Exon 21	c.3897A>G	4029A/G	2	3	7(12/9%)
Exon 4	c.328G>C	p.D110H	1	1	3(5.55%)

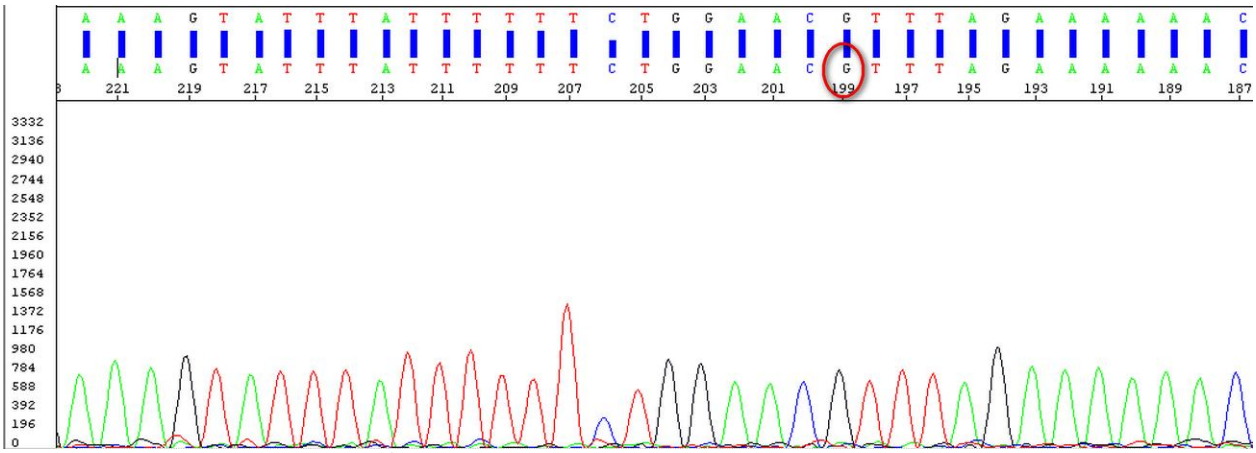

Figure 1. The sequencing results of the CFTR gene illustrating the homozygous polymorphism 4020A/G in exon 21

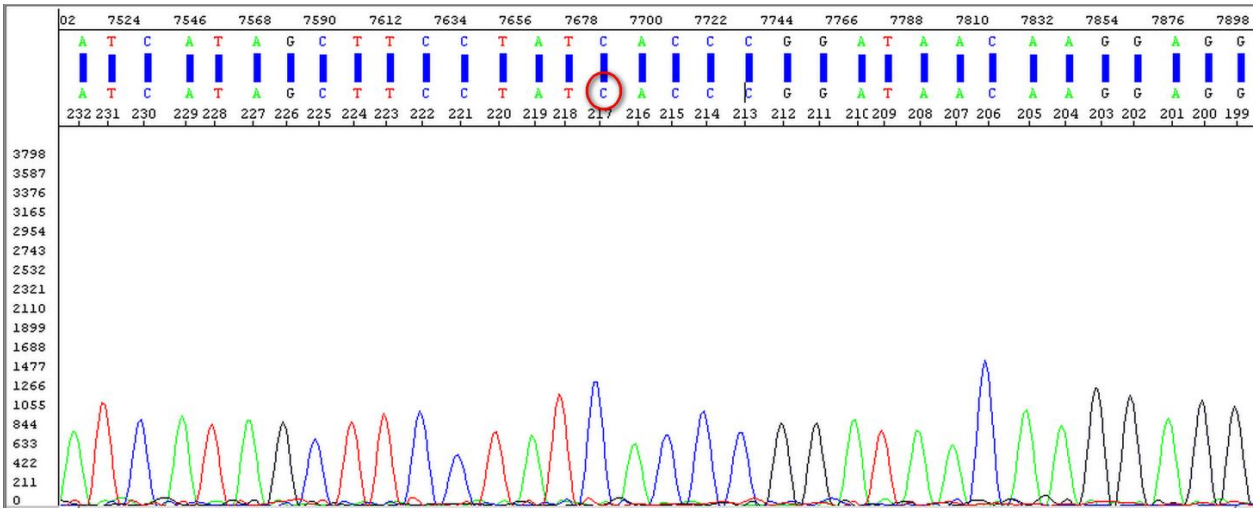

Figure 2. The sequencing results of the CFTR gene showing the heterozygous mutation D110H in exon 4

Discussion

CF is a disease with an autosomal recessive pattern, which is commonly found in children. The frequency and distribution of CF mutations vary depending on geographical region and the ethnic and religious background [7]. Despite the lack of accurate statistics for the prevalence of CF in Iran, the limited number of studies conducted estimate the prevalence to be between those in Southern Europe and East Asia, suggesting the need for conducting further studies on this disease in Iran.

Different environmental and genetic factors contribute to the clinical demonstrations of CF in different populations. Nutritional problems and abnormal stool were observed at diagnosis in 80% of the present study patients. Moreover, all the patients suffered mild to severe respiratory problems. According to the Cystic Fibrosis Center in North America, 40.3% of CF patients present nutritional problems at diagnosis, 48.8% respiratory symptoms and 32.2% steatorrhea. Similar to other studies, the present research shows a different pattern of the disease demonstration in Iran and the Middle East [8-9]. The mean age at diagnosis was 52 months in the present study, which is higher than that in the US (6 months), while the mean age of onset of clinical symptoms was 18 months, suggesting a large gap and the need for managing CF in Iran. Furthermore, 66% of the patients were found to have consanguineous parents, while the rate of consanguineous marriage has been reported as 38% in Kurds, which is not a surprising finding given the autosomal recessive pattern of the disease.

The most globally frequent mutations such as ΔF508, G542X, G551D, N1303K and W1282X significantly differ in frequency depending on geographical region and ethnic background. For example, the frequency of ΔF508 is 88% in Danes, 30% in Ashkenazi Jews and 18% in Iranians [10-12]. In addition, rare mutations may become patented in a geographical region. For instance, the frequency of c.2988+1G>A is 8.8%-14% in African-American patients, 2.2%-5.16% in the Spanish and 0.08%-1% in the Caucasian population, while that of Y1092X is 37% in Iraqi Jews. Research suggests low relative frequency for ΔF508 in Iran, Asia and Southern Europe. Moreover, D110H is a rare mutation in the CFTR gene, which was discovered in patients with mild phenotypes [13]. The 110 residue in the CFTR protein plays a key role in the channel stability of the external cavity through interacting with … residues. Mutations in this amino acid thus disrupt the membrane stability [14]. The frequency of the D110H mutations was reported as 17% in Hungary, 34% in Italy and 1.64% in Turkey [15-16]. Elahi et al. also observed this mutation in Iranians [17]. The frequency of the D110H mutation found in the present study is the highest compared to in literature; however, the patients with this mutation did not need

pancreatic enzymes. Historically, this mutation seems to have been transmitted from Northern Europe through Turkey to its neighboring province, Kermanshah, and enriched in its population. The frequency of the rare polymorphism, 4029 A/G, was found to be as high as 12.9% (7 alleles) in the present study, including 4 homozygotes and 3 heterozygotes. This polymorphism is associated with a synonymous threonine substitution at position 1299 of the CFTR gene in the NBD2 domain of the CFTR protein. Synonymous polymorphisms are less frequent in the CFTR gene compared to in other genetic diseases, which may be explained by the strong linkage disequilibrium of the genomic region despite its unknown etiology. A study conducted in 1992 was the only one reporting the observed polymorphism in CF patients [18]. The present study found no mutations in exons 11 and 21, the frequency of which is below 5% in different countries (Table 3). Many studies suggest significant variations in the mutation distribution of exons 11 and 21 in different populations. The frequency of the originally old G542X, as the most important mutation in exon 11, is 8.1% in Spain and 4% in Italy. It also shows an increase in some regions such as Belgium owing to the founder effect caused by immigration from Southern Europe. Furthermore, the frequency of the most important and old mutation in exon 21, N1303K, significantly varies in different countries across the world and in different ethnic backgrounds, ranging from 4.8% in Italy to 17.2% in Tunisia [19], with the Mediterranean and Southern Europe being generally dominant.

Table 3. Comparing the frequency of common mutations G542X and N1303K in Europe, North Africa, Iran and its neighboring counties

Mutation	Region										
	Southwest Iran	Mazandaran, Iran	East Azarbaijan, Iran	Iran	India	Saudi Arabia	Turkey	Lebanon	Pakistan	Europe and North Africa	The present study
G542X	2%	0	2.5%	1.6%-3.6%	0	0	2.6%-4.9%	1.3%	0	2.6%	0
N1303K	2%	0	0	3.4%-5.5%	0	3%	2.9%-3.7%	20%	0	1.6%	0
Reference	[28]	[27]	[26]	[10, 17 and 25]	[24]	[23]	[21-22]		s(20)	[4]	

The limited number of molecular analyses in Iran suggest differences in the distribution of CF mutations between Iranian and Caucasian populations; e.g. although ΔF508 may not be common in Iranian ethnicities, they possess mutations, as do some Asian populations such as Arabs, which are rare or lacking in Caucasians. Moreover, a recently conducted study in Oman identified rare and new mutations and found S549R with a frequency of 65% to be the most common mutation followed by ΔF508 with a frequency of 15% [29]. Similarly, the mutation pattern may be different and specific to the population of Kermanshah. Furthermore, given the high number and low frequency of mutations in the CFTR gene, failing to identify common mutations in exons 11 and 21 including G542X, G553X, N1303K and G551D is justified. The analysis of the PAH gene mutations suggested different mutation spectrum in phenylketonuria patients in Kermanshah province compared to the results of similar studies in Iran and the neighboring countries [30].

Conclusion

The data obtained in the present study suggest rare, new and population-specific mutation patterns in the study population. Given the high frequency of the D110H mutation, other exons are required to be investigated to identify Iranian rather than European mutations. Mutation screening of D110H is also recommended to be conducted in CF patients in Kermanshah province.

Acknowledgement

The authors would like to express their gratitude to the staff of the medical genetics laboratory in Kermanshah University of Medical Sciences as well as to the patients and their parents who consented to the study participation.

References

1. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. Science. 1989;245(4922):1073-80.
2. Vankeerberghen A, Cuppens H, Cassiman JJ. The cystic fibrosis transmembrane conductance regulator: an intriguing protein with pleiotropic functions. J Cyst Fibros. 2002;1(1):13-29.
3. Rowntree RK, Harris A. The phenotypic consequences of CFTR mutations. Ann Hum Genet. 2003;67(Pt 5):471-85.
4. Mateu E, Calafell F, Ramos MD, Casals T, Bertranpetit J. Can a place of origin of the main cystic fibrosis mutations be identified? Am J Hum Genet. 2002;70(1):257-64.
5. Castellani C, Cuppens H, Macek M, Cassiman JJ, Kerem E, Durie P, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros. 2008;7(3):179-96.
6. Sahami A, Alibakhshi R, Ghadiri K, Sadeghi H. Mutation analysis of exons 10 and 17a of CFTR gene in patients with cystic fibrosis in Kermanshah province, western Iran. J Reprod Infertil. 2014;15(1):49-56.
7. Dawson K, Frossard P. The geographic distribution of cystic fibrosis mutations gives clues about population origins. Eur J Pediatr. 2000;159(7):496-9.

8. Khatami GR, Mir-Nasseri MM, Seyghali F, Allah-Verdi B, Yourdkhani F. Characteristics of Patients with Cystic Fibrosis: Experience in a Large Referral Children's Hospital in Tehran, Iran. Middle East J Dig Dis. 2010;2(1):20-3.
9. Naguib ML, Schrijver I, Gardner P, Pique LM, Doss SS, Zekry MAA, et al. Cystic fibrosis detection in high-risk Egyptian children and CFTR mutation analysis. J Cyst Fibros. 2007;6(2):111-6.
10. Alibakhshi R, Kianishirazi R, Cassiman J-J, Zamani M, Cuppens H. Analysis of the CFTR gene in Iranian cystic fibrosis patients: identification of eight novel mutations. J Cyst Fibros. 2008;7(2):102-9.
11. Palomaki GE, Haddow JE, Bradley LA, Fitzsimmons SC. Updated assessment of cystic fibrosis mutation frequencies in non-Hispanic Caucasians. Genet Med. 2002;4(2):90-4.
12. Rohlfs EM, Zhou Z, Heim RA, Nagan N, Rosenblum LS, Flynn K, et al. Cystic fibrosis carrier testing in an ethnically diverse US population. Clin Chem. 2011;57(6):841-8.
13. Monaghan K, Highsmith W, Amos J, Pratt V, Roa B, Friez M, et al. Genotype-phenotype correlation and frequency of the 3199del6 cystic fibrosis mutation among I148T carriers: results from a collaborative study. Genet Med. 2004;6(5):421-5.
14. Cui G, Rahman KS, Infield DT, Kuang C, Prince CZ, McCarty NA. Three charged amino acids in extracellular loop 1 are involved in maintaining the outer pore architecture of CFTR. J Gen Physiol. 2014;144(2):159-79.
15. Křenková P, Piskáčková T, Holubová A, Balaščaková M, Krulišová V, Čamajová J, et al. Distribution of CFTR mutations in the Czech population: Positive impact of integrated clinical and laboratory expertise, detection of novel/de novo alleles and relevance for related/derived populations. J Cyst Fibros. 2013;12(5):532-7.
16. Onay T, Topaloglu O, Zielenski J, Gokgoz N, Kayserili H, Camcioglu Y, et al. Analysis of the CFTR gene in Turkish cystic fibrosis patients: identification of three novel mutations (3172delAC, P1013L and M1028I). Hum Genet. 1998;102(2):224-30
17. Elahi E, Khodadad A, Kupershmidt I, Ghasemi F, Alinasab B, Naghizadeh R, et al. A haplotype framework for cystic fibrosis mutations in Iran. J Mol Diagn. 2006;8(1):119-27.
18. Tsui L-C, Dorfman R. The cystic fibrosis gene: a molecular genetic perspective. Cold Spring Harb Perspect Med. 2013;3(2):a009472.
19. Casals T, Nunes V, Palacio A, Giménez J, Gaona A, Ibáñez N, et al. Cystic fibrosis in Spain: high frequency of mutation G542X in the Mediterranean coastal area. Hum Genet. 1993;91(1):66-70.
20. Shah U, Frossard P, Moatter T. Cystic fibrosis: defining a disease under-diagnosed in Pakistan. Trop Med Int Health. 2009;14(5):542-5.
21. Hundrieser J, Bremer S, Peinemann F, Stuhrmann M, Hoffknecht N, Wulf B, et al. Frequency of the F508 deletion in the CFTR gene in Turkish cystic fibrosis patients. Hum Genet. 1990;85(4):409-10.
22. Yılmaz E, Erdem H, Özgüç M, Coskun T, Özçelik U, Göçmen A, et al. Study of 12 mutations in Turkish cystic fibrosis patients. Hum Hered. 1995;45(3):175-7.
23. Banjar H, Kambouris M, Meyer B, Al-Mehaidib A, Mogarri I. Geographic distribution of cystic fibrosis transmembrane regulator gene mutations in Saudi Arabia. Ann Trop Paediatr. 1999;19(1):69-73.
24. Akhavan-Niaki H, Derakhshandeh-Peykar P, Banihashemi A, Mostafazadeh A, Asghari B, Ahmadifard M-R, et al. A comprehensive molecular characterization of beta thalassemia in a highly heterogeneous population. Blood Cells Mol Dis. 2011;47(1):29-32.
25. Jalalirad M, Houshmand M, Mirfakhraie R, Goharbari M, Mirzajani F. First Study of CF Mutations in the CFTR Gene of Iranian Patients: Detection of F508, G542X, W1282X, A120T, R117H, and R347H Mutations. J Trop Pediatr. 2004;50(6):359-61.
26. Bonyadi M, Omrani O, Rafeey M, Bilan N. Spectrum of CFTR gene mutations in Iranian Azeri Turkish patients with cystic fibrosis. Genet Test Mol Biomarkers. 2011;15(1-2):89-92.
27. Dooki M-RE, Akhavan-Niaki H, Juibary AG. Detecting Common CFTR Mutations by Reverse Dot Blot Hybridization Method in Cystic Fibrosis; First Report from Northern Iran. Iran J Pediatr. 2011;21(1):51-7.
28. Farjadian S, Moghtaderi M, Kashef S, Alyasin S, Najib K, Saki F. Clinical and genetic features in patients with cystic fibrosis in southwestern Iran. Iran J Pediatr. 2013;23(2):212-5.
29. Al-Kindy H, Ouhtit A, Al-Salmi Q, Al-Bimani M, Al-Nabhani M, Gupta I. Novel Mutation in the CFTR Gene of Cystic Fibrosis Patients in Oman. J Mol Biomark Diagn. 2014;5:168.
30. Alibakhshi R, Moradi K, Mohebbi Z, Ghadiri K. Mutation analysis of PAH gene in patients with PKU in western Iran and its association with polymorphisms: identification of four novel mutations. Metab Brain Dis. 2014;29(1):131-8.