REVIEW

doi: 10.5455/medarch.2019.73.118-120

MED ARCH. 2019 APR; 73(2): 118-120

RECEIVED: JAN 12, 2019 | ACCEPTED: FEB 20, 2019

¹Immunology Department, Prince Iman Center, Amman, Jordan²Hematology Department, Prince Iman Center, Amman, Jordan³Internal Medicine, King Hussein Medical Center, Amman, Jordan⁴Pediatric Department, King Hussein Medical Center, Amman, Jordan

Corresponding author: Sanaa, Al-Saeidat, MD, senior specialist, Hematology Department, Prince Iman center, Amman, Jordan, E-mail: dr.sanaasaidat@yahoo.com. ORCID ID: <http://www.orcid.org/0000-0002-8223-5748>.

Cystic Fibrosis Gene Mutation Frequency Among a Group of Suspected Children in King Hussein Medical Center

Baheieh Al-abadi¹, Mansour Al-hiary¹, Rami Khasawneh², Asim Al-momani², Ahmad Bani-Salameh³, Sanaa Al-Saeidat², Alia Al-khlaifat⁴, Omaima Abo-alsondos¹

ABSTRACT

Introduction: Cystic fibrosis (CF) is a genetic multisystem disorder that affects mostly the lungs, but other organs such as liver, pancreas and intestine also affected. CF is inherited in an autosomal recessive manner and occurs in males and females equally. Cystic fibrosis Transmembrane Conductance Regulator (CFTR) mutations are classified into five classes. Class 1 (non-functional protein), class 2 (near-absence of mature CFTR protein at the apical cell membrane), class 3 (full-length CFTR protein incorporated into the cell membrane), class 4 (reduced conductance CFTR mutation), and class 5 (reduced amount of CFTR protein with normal function). Globally F508 mutation is the most common. **Aim:** The aim of this study was to determine the frequency of CFTR gene mutation in Jordanian populations attending a major hospital (KHMC). **Material and Methods:** This is a retrospective study was conducted on 777 sera samples for patients clinically suspected to have cystic fibrosis over a six year period 1/1/2013-1/10/2018. The patient's age range between 1year and 33 years, of which 59.2% (460) were male and 40.8% (317) female. Blood samples were analyzed at Princess Iman Centre for Research and Laboratory Sciences at King Hussein Medical Centre. The samples were tested for 34 mutations of CFTR gene using CF Strip Assay VIENNA LAB Diagnostics GmbH, Austria by polymerase chain reaction (PCR). **Results:** A total of 777 patients samples were analyzed for cystic gene mutations. Twelve (12) mutations were identified. In 49 patients (6.3%) were heterozygous genotype mutant and 28 (3.6%) were homozygous. The most frequent mutation F508del was found in 32/77 (41.5%). 20 (25.9%) of them were heterozygous genotype mutant and 12 (15.6%) were homozygous genotype mutant. The second frequent mutation was N1303K with frequency rate 15.6% (12/77), 9 (11.7%) of them were heterozygous and 3 (3.9%) were homozygous. Regarding frequency of cystic fibrosis gene mutation depending on sex, 55.8% (43/77) of mutations were found in male, whereas 44.2% (34/77) in female. **Conclusion:** Our findings suggest that cystic fibrosis in Jordan is not a rare disease, and found that the most frequent CFTR gene mutation was F508del, which is in keeping with results from other Mediterranean countries.

Keywords: Cystic fibrosis, CFTR gene, mutation genotype.

1. INTRODUCTION

Cystic fibrosis (CF) is a genetic multisystem disorder that affects mostly the lungs, but other organs such as liver, pancreas and intestine also affected. CF is inherited in an autosomal recessive manner and occurs in males and females equally (1). The disease is caused by a mutation in cystic fibrosis transmembrane conductance regulator (CFTR) gene that encode CFTR protein, which play important role for transporting chloride ions through membranes and lead to increased viscosity of the mucous in the lung, liver, pancreas and intestines (2). As a result the

symptoms of cystic fibrosis includes chronic obstructive pulmonary disease (infections with *Pseudomonas aeruginosa* and *Staphylococcus aureus*), liver and pancreatic fibrosis (poor nutrient uptake and pancreatic damage that can lead to diabetes mellitus) (3). Cystic fibrosis distributed worldwide and most common (1 in 2500 white individuals) in North Europe and 1 in 2270 Ashkenazi Jews (4). The cystic fibrosis gene, CFTR was identified in 1989 by Riordan et al and Rommens et al (5), and it is located on the seventh human chromosome at the position 7q13 (6). Regarding CFTR gene mutation there

© 2019 Baheieh Al-abadi, Mansour Al-hiary, Rami Khasawneh, Asim Al-momani, Ahmad Bani-Salameh, Sanaa Al-Saeidat, Alia Al-khlaifat, Omaima Abo-alsondos

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

are 1900 mutations were identified, of which 1500 are potentially cause of cystic fibrosis in patients suspected to have the disease. CFTR mutations are classified into five classes. Class 1 (non-functional protein), class 2 (near-absence of mature CFTR protein at the apical cell membrane), class 3 (full-length CFTR protein incorporated into the cell membrane), class 4 (reduced conductance CFTR mutation), and class 5 (reduced amount of CFTR protein with normal function) (7). Globally F508 mutation is the most common (6, 8, 9). Diagnosis of cystic fibrosis performed using CFTR molecular testing that is based on direct gene analysis procedures. There are two groups of methods for this purpose: first one is method for analysis of samples DNA for presence or absence of specific mutations, and the second is screening samples for any deviation from the standard sequence (10).

2. AIM

The aim of this study was to determine the frequency of CFTR gene mutation in Jordanian populations attending a major hospital (KHMC).

3. MATERIAL AND METHODS

Our publication was approved by the research ethics committee of the Royal Medical Services, Amman-Jordan. This is a retrospective study was conducted on 777 sera samples for patients clinically suspected to have cystic fibrosis over a six year period 1/1/2013-1/10/2018. The patient's age range between 1 year and 33 years, of which 59.2% (460) were male and 40.8% (317) female. Blood samples were analyzed at Princess Iman Centre for Research and Laboratory Sciences at King Hussein Medical Centre. The samples were tested for 34 mutations of CFTR gene using CF Strip Assay VIENNA LAB Diagnostics GmbH, Austria by polymerase chain reaction (PCR). DNA was isolated from blood, amplified using biotinylated primers, then hybridization of amplification products to strips containing allele-specific probes immobilized as array of parallel lines, and finally bound sequences were detected using streptavidin-alkaline-phosphatase and color substrate. For each sample 2 strips was used, strip A that cover 18 mutations, and strip B that cover 16 mutations. Each test strip contains a positive reaction of the uppermost control line that indicates the correct function of conjugate solution and color developer.

4. RESULTS

A total of 777 patients samples were analyzed for cystic gene mutations. Twelve (12) mutations were identified. These mutations were F508del, G85E, N1303K, A455E, W1282X, 2184delA, 2183AA>G, 3849, R117H, 2789+5G>A, R334W, and 3272-26A. In 49 patients (6.3%) were heterozygous genotype mutant and 28 (3.6%) were homozygous. The most frequent mutation F508del was found in 32/77 (41.5%). 20 (25.9%) of them were heterozygous genotype mutant and 12 (15.6%) were homozygous genotype mutant. The second frequent mutation was N1303K with frequency rate 15.6% (12/77),

Mutation	Heterozygous gene mutant		Homozygous gene mutant		Total
	Number	Percentage	Number	Percentage	
F508del	20	25.9%	12	15.6%	32(41.5%)
N1303K	9	11.7%	3	3.9%	12(15.6%)
G85E	5	6.5%	4	5.2%	9(11.7%)
W1282X	3	3.9%	3	3.9%	6(7.8%)
A455E	5	6.5%	0	0%	5(6.5%)
2183AA>G	2	2.6%	3	3.9%	5(6.5%)
2184delA	1	1.3%	0	0%	1(1.3%)
3849	2	2.6%	0	0%	2(2.6%)
2789+5G>A	0	0%	2	2.6%	2(2.6%)
R334W	0	0%	1	1.3%	1(1.3%)
3272-26A	1	1.3%	0	0%	1(1.3%)
R117H	1	1.3%	0	0%	1(1.3%)
Total	49	63.6%	28	36.4%	77(100%)

Table 1. Frequency of cystic fibrosis gene mutations.

9 (11.7%) of them were heterozygous and 3 (3.9%) were homozygous Table 1. Regarding frequency of cystic fibrosis gene mutation depending on sex, 55.8% (43/77) of mutations were found in male, whereas 44.2% (34/77) in female. 3272-26A and R334W mutations were found only in female patients with 1.3% frequency rate for each. Male were more frequently had F508del mutation (21/43-48.8%) in comparison with female, whose more frequently had F508del mutation (11/34-32.4%) N1303K (7/34-20.5%) and G85E (5/34-14.7%) (Figure 1).

5. DISCUSSION

Our study examined a 777 of Jordanian patients with clinical features suspected to have cystic fibrosis for 34 mutations of CFTR gene. In Jordan the first report about cystic fibrosis was in 1984 by Nazer HM (11). Our results shows that F508del mutation was the most frequent (41.5%), followed by N1303K (15.6%) and G85E (11.7%). Study conducted by F. Al Sheyab et al for 3 mutations (F508del, W1282X, N1303K) report that frequency of F508del mutation was 23.75%, W1282X mutation (15%) and N1303K not detected. Lowest frequency of F508del may be due to a small number of specimens used in their study (120) in comparison with our study (777) (12). The frequency of W1282X in our study was 7.8%. In the surrounding countries such as Palestine Laufer-Cahana A et al report that the mutations DF508, N1303K, W1282X were found in all Arab ethnic subgroups, which is in keeping with our results (13). In Lebanon study conducted by Farra C shows F508del mutation was the most common with 34% frequency rate (14). Comparing our results with those from western Arab countries such as in Algeria and Tunisia, most frequent mutation in Algeria reported by Fatima Zohra Sediki was F508del (18.75%) and this lower frequency in comparison with our results due to small number of sample size (24 families). In Tunisian population the frequency of F508del was 50.4% (15). In European population the most frequent mutation is F508del with frequency rate ranged between 43% and 87.2% (9). Studies from Latin Amer-

Figure 1. Cystic fibrosis gene mutation in male and female.

ica show that F508del was most common in Venezuela (27.7%) (16) and 66.7% in Brazil (17). As we mentioned earlier the variation in frequency rate of mutation may be due to a variation in sample size in each study, but our findings suggest that F508del was the most frequent.

6. CONCLUSION

Our findings suggest that cystic fibrosis in Jordan is not a rare disease, and found that the most frequent CFTR gene mutation was F508del, which is in keeping with results from other Mediterranean countries.

- **Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms.
- **Authors' contributions:** Each author gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work. Each author had role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
- **Conflicts of interest:** There are no conflicts of interest
- **Financial support and sponsorship:** None

REFERENCES

1. James CC, Lynn MT. An Introduction to Cystic Fibrosis for Patients and Their Families 6th ed. 2013.
2. Mohammad RM, Jamal F, Mohsen R. et al. Cystic fibrosis prevalence among a group of high-risk children in the main referral children hospital in Iran. *Journal of Education and Health* Promotion. 2017; 6(54).
3. Health Council of the Netherlands. Neonatal screening for cystic fibrosis, 2010.
4. Schrijver I. Mutation Distribution in Expanded Screening for Cystic Fibrosis: Making Up the Balance in a Context of Ethnic Diversity. *Clinical Chemistry*. 2011; 57(6): 799-801.
5. Joseph LB, Milan MJ, Jason PF et al. Cystic Fibrosis: A Worldwide Analysis of CFTR Mutations. Correlation with Incidence Data and Application to Screening. *Human Mutation*. 2002; 19: 575-606.
6. Karnajit KB, Arup KM, Prosenjit P. et al. Allele frequency for Cystic fibrosis in Indians vis-a-vis global populations. *Biomedical Informatics*. 2015; 11(7): 348-352.
7. Boeck KD, Zolin A, Cuppens Het al. The relative frequency of CFTR mutation classes in European patients with cystic fibrosis. *Journal of Cystic Fibrosis*. 2014; 13: 403-409.
8. Saima B, Safia F, Munir AL et al. Pattern of cystic fibrosis based on sweat chloride test in children- a hospital based analysis. *Pakistan Journal of Pathology*. 2017; 28(2): 85-91.
9. Oscar L, Aida MA, Eva M. et al. Spatial patterns of cystic fibrosis mutation spectra in European populations. *European Journal of Human Genetics*. 2003; 11: 385-394.
10. Els D, Manfred S, Michael AM. et al. Best practice guidelines for molecular Genetic diagnosis of cystic fibrosis and CFTR-related disorders—updated European recommendations. *European Journal of Human Genetics*. 2009; 17: 51-65.
11. Nazer HM. Early diagnosis of cystic fibrosis in Jordanian children. *J Trop Pediatr*. 1992 Jun; 38(3): 113-115. doi: 10.1093/tropej/38.3.113.
12. Al Sheyab F, Ballat SM, Rawashdeh M. Relative Frequencies of Three Cystic Fibrosis Mutations in North Jordan; "F508, W1282X, and N1303K. *Int J Hum Genet*. 2007; 7(2): 137-140.
13. Laufer A, Lerer I, Sagi M. et al. Cystic fibrosis mutations in Israeli Arab patients. *Hum Mutat*. 1999 Dec; 14(6): 543.
14. Farra C, Menassa R, Awwad J. et al. Mutational spectrum of cystic fibrosis in the Lebanese population. *J Cyst Fibrosis*. 2010 Dec; 9(6): 406-410. doi: 10.1016.
15. Fatima ZS, Abdelkarim R, Faiza C. et al. Detection of CFTR mutations using PCR/ARMS in a sample of Algerian population. *Ann Biol Clin*. 2014; 72(5): 549-554.
16. Karen S, Elizabeth M, Xiorama M. et al. Analysis of the CFTR gene in Venezuelan cystic fibrosis patients, identification of six novel cystic fibrosis-causing genetic variants. *The Application of Clinical Genetics*. 2016; 9: 33-38.
17. Carmen SL, Manoela MO, Fernando AL. et al. Cystic fibrosis transmembrane conductance regulator gene mutations and glutathione S-transferase null genotypes in cystic fibrosis patients in Brazil. *J Bras Pneumol*. 2012; 38(1): 50-56.