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Correlation of Cystic Fibrosis Mutations (CFTR) and Bacteria in a Tertiary Care Center in Saudi Arabia

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Abstract

Introduction*: Bacterial infections in CF patients are common and start early in life. The prognosis of the disease is substantially dependent on chronic respiratory infection and inflammation. The correlation between cystic fibrosis transmembrane regulator gene mutations (CFTR) and bacterial colonization is poorly understood.*

Objectives: *To obtain the correlation of CFTR mutations and types of bacterial cultures in CF patients in tertiary care center Saudi Arabia.*

Methodology: *A retrospective study of 304 patients with confirmed CF of all age groups who had respiratory samples with positive culture at presentation and at last follow-up period from 1st January 1989- 30 December 2018.*

Results: *A total of 304 patients had respiratory cultures obtained at a presentation at a mean age of 4.8 (SD 5.9) years. A total of 262 patients had first respiratory culture, in addition to CFTR identification. The most common mutations in descending frequency were: (p.G473EfsX54 in 49 (18.97%), p.I1234V in 32 (12.21), 711+1G>T in 30 (11.45), p.Phe508del in 29 (11.07), 3120+1G>A in 22 (8.7), p.His139Leu in 22 (8.40), p.Gln637Hisfs in 14 (5.34), p.Ser549Arg in 9 (3.44), p.N1303 K in 7 (2.67) and other mutations in 48 (18.32) in descending frequency.*

There was no difference in the frequencies of all types of CFTR mutations in both the first and last respiratory cultures at a mean age of 17.80 (SD 7.17) years (p=0.33). There was a persistent increase in the prevalence of all types of Pseudomonas cultures, and a decrease in the prevalence of both Staphylococcus aureus and Haemophilus influenza bacteria in all types of CFTR mutations (P= >0.05).

Conclusion: *There is a progressive increase in the number of patients with the most pathogenic types of bacteria (Pseudomonas aeruginosa) in all types of CFTR mutation at presentation and continued through the follow-up period. There is a need for awareness of early eradication of pathogenic bacteria to prevent progressive pulmonary damage.*

Keywords: *cystic fibrosis, bacteriology, microbiology, CFTR, Arab.*

Abbreviations:

CF: Cystic fibrosis

PFT: Pulmonary function test

P. aeruginosa: Pseudomonas aeruginosa

S. aureus: Staphylococcus Aureus

B. cepacia complex: Burkholderia cepacian complex

H. influenzae: Haemophilus influenza

MRSA: Methicillin-resistant Staphylococcus aureus

MSSA: Methicillin-sensitive Staphylococcus aureus

BAL: Bronchoalveolar lavage

NPA: Nasopharyngeal aspirate

CFTR: Cystic fibrosis Transmembrane conductance Regulator

N: Number

SD: Standard deviation

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive lethal hereditary disorder in Caucasians **(**1**)**. The prognosis of the disease is substantially dependent on chronic respiratory infection and inflammation, a hallmark of CF **(**2**)**. Pseudomonas aeruginosa (P. aeruginosa) is the dominant pathogen in patients with CF **(**2**)**. The median survival age of individuals with CF in industrialized countries increased from 14 years in 1969 to more than 30 years in 2001 and approximately 37% of patients are 18 years of age or older **(**2**)**. European registries report similar median survival ages **(**3**)** and there is evidence that disease severity is determined by the genetic mutations present. **(**4**)**.

During the last 5 years, reported survival rates appear to have reached a plateau in some industrialized countries **(**2**)**. Strategies to substantially increase life expectancy in CF include a neonatal screening of the general population to identify CF, early initiation of antimicrobial and anti-inflammatory therapy in identified patients, implementation of effective hygienic measures inside and outside of CF centers, and, establishment of patient registries **(**5**)**.

Microbiological data has been reported before in Saudi Arabia in a small sample of patients **(**6**)**. The most common organism was Staph aureus, Hemophilus influenza and Pseudomonas aeruginosa. Patients became colonized with Pseudomonas aeruginosa at an earlier age of 3 years compared to 7 years in other reports **(**6,7**).**

In the United States, cystic fibrosis (CF) affects roughly 30,000 people, reducing life expectancy by 50%. Progressive pulmonary disease, marked by recurrent exacerbations, bacterial infection, and declining lung function, drives morbidity and mortality **(**6-8**).** Studies of the CF lung reveal diverse microbiology. Methicillin-sensitive Staphylococcus aureus (MSSA) and Pseudomonas aeruginosa are the two organisms most commonly isolated from the airway **(**8**)**. Opportunistic organisms, including Burkholderia cepacia complex, Stenotrophomonas maltophilia, Achromobacter xylosoxi-dans, nontuberculous mycobacteria, and fungal organisms, commonly colonize and infect patients with CF.

In this report, we would like to elaborate on the relation of bacterial colonization to the type of cystic fibrosis transmembrane regulator gene mutation (CFTR).

Aim of the study

To obtain the correlation of CFTR mutations and type bacterial cultures in CF patients in tertiary care center Saudi Arabia.

Methodology

After obtaining ethical approval, we retrospectively reviewed the charts of 304 patients with confirmed CF of all age groups who had respiratory samples with positive culture on regular follow-up or during respiratory exacerbation from the period 1st January 1989- 30 December 2018.

Definition: Patients with CF are defined as those who have typical pulmonary manifestations and/or typical gastrointestinal manifestations (GI) and/or a history of CF in the immediate family in addition to a sweat chloride concentration of 60 mmol/L or if they have the pathologic CFTR mutations on both chromosomes **(**8,9**)**.

Inclusion Criteria: All confirmed CF patients of all the age groups that had respiratory cultures results (positive or negative) during their follow-up period in CF clinic from the period 1989- 30 December 2018.

Types of samples: Nasopharyngeal aspirates (NPA) were collected from patients who were unable to expectorate below the age of 4 years. Induced sputum samples were obtained from patients above 4 years of age. Bronchoalveolar lavage (BAL) samples were collected from patients with severe CF pulmonary disease. Cultures were repeated every 3-6 months during the follow-up period.

Method of sample collection: Bronchoalveolar lavage and NPA samples were collected for bacterial cultures and processed according to standard methodology [6-8]. Samples were collected following standard hospital precautions.

CFTR identifications: As mentioned before in a previous publication from our center **(**9**)**.

Ethical considerations: The Declaration of Helsinki and good clinical practice guidelines were followed. Data collection and data entry were supervised by the principal investigator. All data needed were obtained by a retrospective chart review. All data were stored in the pediatrics research unit, accessed only by the principal investigator and the assigned Clinical Research Coordinator. The entire patient's information is kept strictly confidential. Each patient was given a study number, and all patients' data

were entered into the designated data sheet (EXCEL) without any patient's identification. The department of Biostatistics Epidemiology and Scientific Computing (BESC) carried out a statistical analysis of the data.

Statistical Method: For continuous variables, mean, standard deviation and median were calculated using T-Test. Results were presented at a level of significance of $p< 0.05$. All values were expressed in mean ± standard deviation (SD).

Results

A total of 304 patients had respiratory cultures obtained at a presentation at a mean age of 4.8 (SD 5.9) years. One hundred and fifteen patients (37.8%) are from the Eastern province, 36 (12%) are from the western province, 77 (25.2%) from the central rea, 40 (13%) from the northern province and 36 (12%) from the southern province.

Respiratory cultures were taken from nasopharyngeal aspiration (NPA) 182 (59.7%), 112 (37%) was induced sputum. Tracheal aspirate in 9 (3%), one patient (0.3%) by BAL (Table 1).

Count	First respiratory culture # (%)	Last FU culture # (%)
Age in years (SD)	4.8 (5.9)	17.80 (7.17)
1A H influ	7 (2.30)	3 (1.16)
1B Staph	62 (20.39)	44 (17.05)
1C Pseudomonas	120 (39.48)	137 (53.11)
other	115 (37.83)	74 (28.68)
Total	304 (100)	258 (100)

P value= 0.0115, SD= Standard deviation, #= number, (%)= percentage, 1A= Haemophilus influenzae, 1B= Staphylococcus aureus, 1C= Pseudomonas aeruginosa.

Table1: Types of bacterial cultures during first and last follow up period (Total 304 patients).

CFTR/ Protein change	Location	Nucleotide change	Accession #	Patients at First culture # (%)	Patients at Last FU culture # (%)
p.G473EfsX54	Exon 11	c.1418delG	rs397508205	49 (18.97)	42 (19.27)
p.I1234V	Exon 22	c.3700A>G	rs75389940	32 (12.21)	26 (11.90)
711+1G>T	Intron 5	c.579+1G>T	rs77188391	30 (11.45)	20 (9.17)
p.Phe508del	Exon 11	c.1521_1523delCTT	rs113993960	29 (11.07)	25 (11.47)
3120+1G>A	Intron 18	c.2988+1G>A	rs75096551	22 (8.7)	19 (10.81)
p.His139Leu	Exon 4	c.416A>T	rs76371115	22 (8.40)	18 (8.26)
p.Gln637Hisfs	Exon 14	c.1911delG	rs1554389296	14 (5.34)	10 (4.59)
p.Ser549Arg	Exon 12	c.1647T>G	rs121909005	9 (3.44)	9 (4.13)
p.N1303K	Exon 24	c.3909C>G	rs121909005	7 (2.67)	7 (3.21)
Other				48 (18.32)	42 (19.27)
Total				262 (100)	218 (100)

p. value= 0.3389, Accession number=refSNP¼ Reference Single Nucleotide Polymorphism Database, https://www.ncbi.nlm.nih.gov/snp

Table 2: Number of patients with positive Bacteria at first culture and last follow up period compared with CFTR types

A total of 262 patients had first respiratory culture, in addition to CFTR identification. The most common mutations in descending frequency were: (p.G473EfsX54 in 49 (18.97%), p.I1234V in 32 (12.21), 711+1G>T in 30 (11.45), p.Phe508del in 29 (11.07), 3120+1G>A in 22 (8.7), p.His139Leu in 22 (8.40), p.Gln637Hisfs in 14 (5.34), p.Ser549Arg in 9 (3.44), p.N1303K in 7 (2.67) and other mutations in 48 (18.32) in descending frequency (Table 2).

Similarly, a total of 218 patients had the last follow-up respiratory culture at a mean age of 17.80 (SD 7.17) years, in addition to CFTR identification. The most common mutations in descending frequency were: (p.G473EfsX54 in 42 (19.27), p.I1234V in 26 (11.90), 711+1G>T in 20 (9.17), p.Phe508del in 25

(11.47), 3120+1G>A in19 (10.81), p.His139Leu in 18 (8.26), p.Gln637Hisfs in 10 (4.59), p.Ser549Arg in 9 (4.13), p.N1303K in 7 (3.21) and other mutations in 42 (19.27) in descending frequency (Table 2).

There was no difference in the frequencies of all mutations in both the first and last respiratory cultures (p=0.33) (Table 2).

CFTR/ Protein change	First FU culture					Last FU culture					P. value
	1A #(%)	1B #(%)	1C #(%)	OTHER #(%)	TOTAL #(%)	1A #(%)	2B #(%)	3C #(%)	Other #(%)	TOTTAL #(%)	
p.G473EfsX54	--	11 (22.9)	19 (37.6)	19 (38.7)	49 (100)	--	6 (14.3)	24 (57.1)	12 (28.6)	42 (100)	0.20
p.I1234V	--	8 (25.0)	14 (43.7)	10 (31.3)	32 (100)	--	4 (15.4)	14 (53.8)	8 (30.8)	26 (100)	0.61
711+1G>T	1 (3.3)	6 (20.0)	8 (26.7)	15 (50.0)	30 (100)	--	5 (25.0)	10 (50.0)	5 (25.0)	20 (100)	0.79
p.Phe508del	2 (7.3)	5 (17.2)	12 (41.3)	10 (34.4)	29 (100)	1 (4.2)	4 (16.7)	13 (54.1)	6 (25.0)	24 (100)	0.17
3120+1G>A	1 (4.5)	3 (13.7)	11 (50.0)	7 (31.8)	22 (100)	--	2 (10.5)	14 (73.7)	3 (15.8)	5 (100)	0.17
p.His139Leu	1 (4.5)	6 (27.3)	5 (22.7)	10 (45.4)	22 (100)	--	3 (16.7)	9 (50.0)	6 (33.3)	18 (100)	0.24
p.Gln637Hisfs	--	1 (7.1)	6 (42.9)	7 (50.0)	14 (100)	--	2 (20.0)	2 (20.0)	6 (60.0)	10 (100)	0.39
p.Ser549Arg	1 (11.1)	2 (22.2)	5 (55.6)	1 (11.1)	9 (100)	1 (11.1)	--	5 (55.6)	3 (33.3)	9 (100)	0.28

p.N1303K	--	3 (42.7)	1 (14.3)	3 (42.9)	7 (100)	--	1 (50.0)	1 (50.0)	--	2 (100)	0.04
other	--	13 (27.1)	16 (33.3)	19 (39.6)	46 (100)	1 (2.4)	10 (23.8)	15 (35.7)	16 (38.1)	42 (100)	0.64
TOTAL CF bacteria	253					218					

A1 = Haemophilus influenza. B1= Staphylococcus aureus. C1= Pseudomonas aeruginosa. #= number. (%)= percentage. CFTR= Cystic fibrosis transmembrane conductance gene.

Table 3: Comparisons of types of pathogenic bacteria from first and last cultures in comparison to different types of CFTR mutations.

In comparing all types of bacterial cultures during the first and the last follow-up cultures from all types of mutations: showed a persistent increase in the prevalence of all types of *Pseudomonas* cultures, and decreased in the prevalence of both Staphylococcus aureus and *Haemophilus* influenza bacteria (P= >0.05) (Table 3).

Discussion

In a French study of CF patients (10) from the registry (2013–2014) studying CF patients aged ≥ 20 years, their clinical outcomes, CF Transmembrane Conductance Regulator (CFTR) genotypes, and microbiological data and carried out a comparison for the positive respiratory culture at least once for *P. aeruginosa* ("Pyo" group, *n* = 1,827), to those patients with no history of *P. aeruginosa* isolation ("Never" group, *n* = 303): The predictive factors of non-colonization by *P. aeruginosa* were identified by multivariate logistic regression model with backward selection. Absence of aspergillosis (odds ratio (OR) [95% CI] = 1.64 [1.01–2.66]), absence of diabetes (2.25 [1.21–4.18]), pancreatic sufficiency (1.81 [1.30–2.52]), forced expiratory volume 1 (FEV1) ≥ 80% (3.03 [2.28–4.03]), older age at CF diagnosis (1.03 [1.02–1.04]), and absence of F508del/F508del genotype (2.17 [1.48–3.19]) were predictive clinical factors associated with absence of infection ("Never" group). Microbiologically, this same group was associated with more frequent detection of *Haemophilus influenzae* and lower rates of *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Aspergillus spp.* (all $p<0.01$) in sputum (40). This study strongly suggests that the absence of pulmonary colonization by *P. aeruginosa* in a minority of CF adults

(14.2%) is associated with a milder form of the disease. Recent progress in the development of drugs to correct CFTR deficiency thus may be decisive in the control of *P. aeruginosa* lung infection **(**10**)**.

Another study of adult CF patients **(**11**)** that were followed for two years and assigned to one of three groups depending on whether they are chronically infected with *Burkholderia cepacia* complex (BCC) organisms, *Pseudomonas aeruginosa*, or neither of these organisms. Genotype analysis was performed on all patients to determine which the cystic fibrosis transmembrane regulator (*CFTR*) gene mutations are present. There were a total of 59 patients: 15 colonized with BCC, 24 colonized with *P. aeruginosa*, and 20 not colonized with either organism. Twenty patients were homozygous for ΔF508, 25 were heterozygous, and the ΔF508 mutation was not present in the remaining 14 patients. Patients homozygous or heterozygous for the ΔF508 mutation had an increased likelihood of colonization with BCC or *P. aeruginosa*, an increased number of positive sputum cultures and a higher frequency of multiple infecting organisms. Cystic fibrosis mutational analysis identified seven patients who had the R117H mutation. These patients were less likely to be colonized with BCC or *P. aeruginosa.* The study concluded that: patients homozygous or heterozygous for the ΔF508 deletion are more likely to suffer airway colonization with BCC or *P. aeruginosa*, with increased numbers of positive sputum cultures and infecting organisms. Those with the R117H mutation are less likely to be colonized by Gram-negative organisms **(**11**)**.

In comparing our study to both studies: The F508del genotype is less common in our population and ranked as the 3rd or the 4th in its frequency of prevalent CFTR mutations **(**9,12**)**. We have reported that the top 3 prevalent mutations in our population were 2 newly described mutations in the Arab population namely (p.G473EfsX54 and 711+1G>T) and the reported mutation (p.I1234V). All 3 mutations had colonization of *P. aeruginosa* at presentation and continued to increase through the follow-up period **(**9,12**)** (Table2, and Table 3). It is worth mentioning that our CFTR mutations are 95% in homozygous state compared to 35-50% in North American or the European CF population (9). The mutation R117H mutation was not common in our population **(**9**)**.

Other factors could have played a role in bacterial colonization, such as delayed eradication of bacteria due to absence of symptoms or healthy-looking CF patients despite pulmonary function or radiological changes that were misinterpreted to have mild changes. Other factors as delayed referral to CF centers and poor compliance to airway clearance techniques and other viral infections **(**13**)**.

Persistence or increase in *P. aeruginosa* colonization during the follow-up period across all types of mutations in our CF population could be explained by many factors, of which poor compliance to anti-pseudomonal inhalation treatment as inhaled Tobramycin or Tobi **(**5**)**.

Further study is needed to determine other factors that may play a role in the persistence of bacterial colonization in different CFTR mutations.

Conclusion

There is a progressive increase in the number of patients with the most pathogenic types of bacteria in all types of CFTR mutation. There is a need for awareness of early eradication of pathogenic bacteria to prevent progressive pulmonary damage.

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References

1.Ratjen F, Do¨ring G. Cystic Fibrosis. Lancet 2003;361:681– 9.

2. “Cystic Fibrosis Foundation Patient Registry 2001 Annual Data Report”. Bethesda (MD), USA: Cystic Fibrosis Foundation; 2002.

3.Stern M, Sens B, Wiedemann B, Busse O. Qualita¨tssicherung Mukoviszidose-U¨ berblick u¨ber den Gesundheitszustand der Patienten in Deutschland 2001. Zentrum fu¨ r Qualita¨tsmanagement im Gesundheitswesen. Germany: A¨ rztekammer Hannover; 2002.

4.Koch C, McKensey SG, Kaplowitz H, Hodson ME, Horms HK, Navarro J, Mastella G. “International practice patterns by age and severity of lung disease in cystic fibrosis: data of the Epidemiologic Registry of Cystic Fibrosis (ERCF)”. Pediatr Pulmonol 1997;24:147– 54.

5.Banjar H., Angyalosi G. “The road for survival improvement of cystic fibrosis patients in Arab countries”, The International Journal of Pediatrics and Adolescent Medicine; June 2015; Volume 2, Issue 2: 47-58.

6.Banjar H. “Microbiological Data of Cystic Fibrosis patients in a tertiary care center in Saudi Arabia”. Kuwait Medical Journal, Sept. 2004; Vol. 36 (3): 179-181

7.Banjar H, Al-Qahtani H, Yasin W, Al-wgait W,Al-Amer H, Raja R, Al-Nakhli A, Karkour K. The first report of Methicillin-resistant Staphylococcus aureus (MRSA) in cystic fibrosis (CF) patients in Saudi Arabia . IJPAM 2020 ; 7 : 186-190 https://doi.org/10.1016/j.ijpam.2019.10.005.

8.Granchelli,a AM,Adler FR,Keogh R , Kartsonaki, C , Cox,D ,Lioua, T, “Microbial Interactions in the Cystic Fibrosis Airway”. Journal of Clinical Microbiology2018;56(8):1-13

9.Banjar HH, Tuleimat L, El Seoudi AA, Mogarri I, Alhaider S, Nizami IY, et al. “Genotype patterns of cystic fibrosis transmembrane conductance regulator gene mutations: a retrospective descriptive study in Saudi Arabia”. Ann Saudi Med 2019; 40(1): 15-24. DOI: 10.5144/0256-4947.2020.15

10.Réchana Vongthilath, Bénédicte Richaud Thiriez,Clémence Dehillotte,Lydie Lemonnier,Alicia Guillien,Bruno Degano,Marie-Laure Dalphin,Jean-Charles Dalphin,Patrick Plésia.Clinical and microbiological characteristics of cystic fibrosis adults never colonized by Pseudomonas aeruginosa: Analysis of the French CF registry. https://doi.org/10.1371/journal.pone.0210201

11.T.E. Mcmanus, D. Beattie, C. Graham, J.E. Moore & J.S. Elborn (2005) “Cystic fibrosis genotype and bacterial infection: a possible connection”, British Journal of Biomedical Science, 62:2, 85-88, DOI: 10.1080/09674845.2005.11732691

12.Hanaa Banjar a, Ibrahim Al-Mogarri, Imran Nizami, Sami Al-Haider, Talal AlMaghamsi, Sara Alkaf, Abdulaziz Al-Enazi, Nabil Moghrabi. Geographic distribution of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in Saudi Arabia, International Journal of Pediatrics and Adolescent Medicine, https://doi.org/10.1016/j.ijpam.2019.12.002.

13.Banjar H , *, Chaballout M, Karkour K, Al-Ghamdi H,Al-Mogarri I, Al-Haider S, Nizami I , Raja R, AlNakhli A ,The prevalence of viral infections in children with cystic fibrosis in a tertiary care center in Saudi Arabia,IJPAM 2020; 7: 83-87 https://doi.org/10.1016/j.ijpam.2019.09.003.