

Analysis of CFTR Gene Variants and clinical presentations in Children with Diffuse Bronchiectasis and Unknown Etiology

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Abstract

Background: Diffuse bronchiectasis is an irreversible abnormal dilation of proximal subsegmental bronchi. The aim was to investigate and compare CFTR gene mutations and clinical presentations in children with idiopathic bronchiectasis.

Materials and Methods: In a cross-sectional study, all children with idiopathic bronchiectasis who were hospitalized from 2019 to 2020 in Tabriz Children's Hospital, Iran, were reviewed. Bronchiectasis confirmation was based on signs, symptoms, and HRCT findings. Data was collected through medical records, medical history, clinical examination, and para-clinical examination. CFTR variants were examined by liquid chromatography, direct sequencing, and multiple probe ligations. Then children were divided into two groups based on variants identified in the CFTR gene and compared in terms of demographic, clinical, and para-clinical findings. Descriptive statistics, Chi-square Tests, and independent samples t-test was used to analyze the data using SPSS software version 22.0.

Results: Out of 21 patients, 0 (47.6%) children were males with a mean age of 9.75 years. Out of 21 children with diffuse bronchiectasis, five clinically significant CFTR-related gene variants were identified (group 1). Other patients either had only single polymorphism or no variants related with CFTR (group 2). Age, FEV1 and sweat test were lower in group 1 than in group 2.

Conclusion

We observed the CFTR variants in heterozygote form in children with diffuse bronchiectasis with a normal or borderline sweat test. Therefore, it is necessary to determine whether DB is a part of CFTR-Related Diseases failing to meet the diagnostic criteria of Cystic fibrosis or a disease independent of Cystic fibrosis.

Key Words: Children, CFTR variants, Cystic fibrosis Diffuse bronchiectasis.

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1- INTRODUCTION

Diffuse bronchiectasis (DB) is the irreversible and abnormal dilation of the proximal subsegmental part of the bronchus. The disease is characterized with chronic sputum producing cough and recurrent lower respiratory tract infection. Most patients present with variable degrees of airway obstruction (1, 2). Bronchiectasis can be either focal or diffuse and is almost always detectable by high-resolution computed tomography (HRCT),(3). According to studies, the annual incidence and prevalence of bronchiectasis are rising (4). The etiology of this disease is not completely understood, but factors such as infectious agents, vascular obstruction, inflammatory and pulmonary diseases, and immunological and genetic parameters, including cystic fibrosis (CF), can lead to bronchiectasis (5, 6).

CF is an autosomal recessive disease resulting from mutations in the *CFTR* gene which is located on the long arm of chromosome 7 (7, 8). The gene is responsible for encoding cyclic-AM-dependent chloride channels(8, 9). Mutations in *CFTR* gene can disrupt the function of these channels, leading to the concentration of mucus and the colonization of various organisms in respiratory tracts (10). There is a wide heterogeneity in the clinical presentation of CF (11, 12). The classic form is characterized with severe symptoms and involvement of one or more organs while the atypical form manifests with mild or incomplete CF phenotype in at least one organ e.g., pancreatitis or CFTR-related disorders (CFTR-RD), (11).

As an etiology for idiopathic bronchiectasis, today there is growing evidence for the role of *CFTR* gene mutations (13). Various studies have reported at least one or two *CFTR* mutations in 5 to 20% of patients (14, 15). However, these mutations have not been

associated with any problems in other organs, which is normally seen in CF. This phenomenon suggests that DB is not a CFTR-RD or DB itself may be a CFTR-RD that does not present all CF diagnostic criteria (16, 17). In 2007, a study was performed by Bienvenu *et al.* on 100 patients with idiopathic bronchiectasis and a normal sweat test to examine the association between *CFTR* gene mutations and the respective protein dysfunction in airways. This study, which was the first report on the association between phenotype and genotype of *CFTR* in a large population of DB patients with a normal sweat test, supported the hypothesis that even a single mutation in the *CFTR* gene could result in pathogenic outcomes of idiopathic bronchiectasis (5). Therefore, this study was conducted to investigate *CFTR* gene alterations in children with idiopathic DB in the Northwestern Iran.

2- MATERIALS AND METHODS

2-1. Study design and population

In a cross-sectional study, all children with idiopathic bronchiectasis who were hospitalized from 2019 to 2020 in Tabriz Children's Hospital, Tabriz University of Medical Sciences, Iran, which is a training, treatment, and referral hospital in the northwest of Iran was reviewed. This study was performed on Azeri Turks, who are members of one of the largest ethnic groups in Iran (11).

2-2. Method

Children under 18 years of age with idiopathic bronchiectasis were recruited using the census method. Bronchiectasis was determined based on clinical signs, symptoms and HRCT findings. Known causes of bronchiectasis were ruled out in children. Immune deficiency disorders were ruled out by the examination of immunoglobulins and antibody responses in serum. Tuberculosis

was ruled out by the phenotypic determination of T-lymphocytes, PPD test, and sputum samples. Allergy, recurrent infection, rheumatic diseases, and Cartagena syndrome were excluded from medical history. The demographic characteristics were gathered from medical records, patients, or their parents, through interviews.

2-3. Measuring

Sweat test was determined by measuring NaCl sweat by nanoduct method, so we expected the results to be 15 mmol / L higher than when only chloride was measured, so the values of less than 45, 45-75 and more than 75 mmol / L were considered normal, borderline and abnormal, respectively. DNA was extracted from the whole blood (5 ml) by salting out according to standard protocols (10, 11). Ten hot spots (exons of 3, 4, 8, 11, 12, 14, 16, 20, 22, and 24) and their adjacent introns in the *CFTR* gene were amplified according to the hot spots in the Azeri-Turkish population by polymerase chain reaction (PCR), (10, 11), and products were screened for *CFTR* gene mutations by liquid chromatography, direct sequencing and multiple ligation probe analyses.

2-4. Intervention

In this study, children were divided into two groups based on variants identified in the *CFTR* gene, as variants were CF-causing mutations or varying clinical consequence (group 1), and variants were polymorphisms (group 2). All variants have been checked through the website (<https://www.cftr2.org/>). The intervention was the providing of blood sample or sputum from patients. In addition, the

spirometry or HRCT was performed on patients.

2-5. Ethical consideration

This study was approved by the Vice Chancellor for Research Ethics Committee of Tabriz University of Medical Sciences (approval number: TBZMED.REC.59476, approval date: 2018), and all participants gave written informed consent. Also, no additional costs were imposed on the patient.

2-6. Inclusion and exclusion criteria

Inclusion criteria included satisfaction of patients or parents to participate in the study, age under 18, Bronchiectasis confirmed using HRCT, normal or borderline sweat test, normal immunological tests, and normal PPD.

2-7. Data Analyses

Descriptive statistics including frequency, percentage, mean, standard deviation, median, minimum and maximum were used to present the demographic data using SPSS software version 22.0. The Chi-square Tests and Independent samples t Test were used to compare items between groups. P-value less than 0.05 were statistically significant.

3- RESULTS

3-1. Demographic characteristics

In this cross-sectional study, 21 children with diffuse idiopathic bronchiectasis were examined with a mean and median age of 9.75 years (± 2.4 SD), and 9.5 years (min-max 6-15), respectively. The ratio of males to females was approximately 1.1. Demographic and clinical characteristics of patients are shown in **Table.1**.

Table-1: Demographic and clinical characteristics of children with diffuse idiopathic bronchiectasis (n=21).

No.	Gender	Age (Year)	Clinical presentation				Recurrent infection	Recurrent sinopulmonary infection	FH of asthma	FH of Cystic Fibrosis	Sweat Test (mmol/L)	Spirometry		Bacterial Colonization		CFTR mutation	Type
			Cough	Sputum	Clubbing	Crackle						FEV1	FVC	Sputum	BAL		
1	M	8	P	N	N	P	N	P	P	28	79	-	N	N	Hetro F508	CF-Causing	
2	F	13	P	P	P	P	N	N	N	30	68	-	Staphylococcus aureus	N	Hetro G542X	CF-Causing	
3	F	10	P	P	N	P	N	N	N	25	92	-	N	N	Hetro R117H-7T	CF-Causing	
4	F	7	P	P	P	P	N	P	N	30	45	-	Pseudomonas	N	Hetro C.12010-11T>G	Variants of varying clinical consequence	
5	F	9	P	N	N	N	N	N	N	55	70	-	N	N	1556V/M470V	Variants of varying clinical consequence	
6	M	14	P	N	P	P	N	P	N	56	69	72	N	N	Hemo M470V	Polymorphism	
7	F	12	P	P	N	N	N	P	N	59	89	-	N	N	Hetro M470V	Polymorphism	
8	M	8	P	P	N	P	N	N	N	46	66	68	N	N	-	-	
9	M	6	P	P	N	P	N	P	N	67	92	-	N	N	N	N	
10	F	16	P	P	P	P	N	P	N	56	64	-	N	N	N	N	
11	F	10	P	N	P	N	N	P	N	58	86	-	N	N	N	N	
12	F	10	P	P	N	N	N	N	N	57	90	-	N	N	N	N	
13	M	15	P	P	P	N	N	P	N	45	78	95	N	N	N	N	
14	F	11	P	P	N	P	N	N	N	33	76	-	N	N	N	N	
15	M	8	P	P	N	P	N	P	N	33	89	-	N	N	N	N	
16	M	8	P	N	N	N	N	N	N	65	91	-	N	N	N	N	
17	M	6	P	P	N	P	N	P	N	67	92	-	N	N	N	N	
18	M	8	P	N	N	P	N	P	N	35	132	-	N	N	N	N	
19	F	11	P	P	P	P	N	P	N	30	88	-	N	N	N	N	
20	M	11	P	P	P	P	P	P	N	46	52	51	N	N	N	N	
21	F	7	P	P	P	P	N	P	N	59	91	-	N	N	N	N	

M: Male; F: Female; N: Negative; P: Positive; FEV1: Forced Expiratory Volume in 1 Second; FVC: Forced vital capacity.

In this study, out of 8 variants identified in the CFTR gene, 5 variants were CF-causing mutations or variants of varying clinical consequence and 3 variants were polymorphisms. All variants have been checked through the website (<https://www.cftr2.org/>). Following the

detection of 8 variants in the CFTR gene, patients were divided into two groups, including group 1 (contains CF-causing mutations or variants of varying clinical consequences), and group 2 (contains of polymorphisms or non-variants). All variants checked using

(<https://www.cftr2.org/>) are shown in the **Table.2**. In this study, no significant difference was observed between the two groups in terms of age and sex (**Table.2**), although most of the patients were women and younger in group 1 than group 2. In terms of clinical symptoms, cough was observed in all patients, followed by sputum excretion, crackling, recurrent sinopulmonary infections, and clubbing of the fingers, respectively. There was no significant difference between the two groups in terms of clinical symptoms (**Table.2**), although the frequency of recurrent sinopulmonary infections was higher in group 2 than in group 1. Comparing the two groups, there was no significant difference in family history of

CF (**Table.2**), although one case was observed in group 1. Sputum culture was positive in 4 patients, three cases were infected with *Pseudomonas aeruginosa* and one case was infected with *Staphylococcus*. The mean sweat test in all patients of group 1 and group 2 was 46.66 ± 14.33 , 33.6 ± 12.13 , and 50.75 ± 12.65 mmol/L, respectively, which was statistically significant between groups 1 and 2 (**Table.2**). Mean FEV1 in all patients, group 1 and group 2, was $80.90\% \pm 18.12$, $70.8\% \pm 17.25$ and $84.06\% \pm 17.71$, respectively, that this difference was not statistically significant between groups 1 and 2 (**Table.2**), although the value of less than 80 (abnormal) was more common in group 1.

Table-2: Comparison of items between two groups.

Variables		Patients with mutations n= 5 (42%)	Patients without mutations n=16 (68%)	Total	P-value	95% OR
Gender	Male	1 (20)	9 (56.3)	10 (47.6)	0.3	5.14 (0.46-56.89)
	Female	4 (80)	7 (43.8)	11 (52.4)		
Cough	P	5 (100)	16 (100)	21 (100)	-	-
	N	0 (0)	0 (0)	0 (0)		
Sputum	P	3 (60)	12 (75)	15 (71.4)	0.5	2 (0.24-16.61)
	N	2 (40)	4 (25)	6 (28.6)		
Clubbing	P	2 (40)	7 (43.8)	9 (42.9)	1	1.16 (0.15-9)
	N	3 (60)	9 (56.3)	12 (57.1)		
Crackle	P	4 (80)	11 (68.8)	15 (71.4)	1	0.55 (0.04-6.26)
	N	1 (20)	5 (31.3)	6 (28.6)		
Recurrent infection	P	0 (0)	1 (6.3)	1 (4.8)	1	0.75 (0.58-0.96)
	N	5 (100)	15 (93.8)	20 (95.2)		
Recurrent sinopulmonary infection	P	2 (40)	12 (75)	14 (66.7)	0.2	4.5 (0.54-37.37)
	N	3 (60)	4 (25)	7 (33.3)		
Asthma	P	0 (0)	1 (6.3)	1 (4.8)	1	0.75 (0.58-0.96)
	N	5 (100)	15 (93.8)	20 (95.2)		
FH of asthma	P	1 (20)	0 (0)	1 (4.8)	0.2	0.2 (0.08-0.48)
	N	4 (80)	16 (100)	20 (95.2)		
FH of Cystic Fibrosis	P	1 (20)	0 (0)	1 (4.8)	0.2	0.2 (0.08-0.48)
	N	4 (80)	16 (100)	20 (95.2)		
Bacterial Colonization	<i>Pseudomonas aeruginosa</i>	1 (20)	2 (12.5)	3 (14.3)	0.2	0.21 (0.02-2.18)
	<i>Staphylococcus aureus</i>	1 (20)	0 (0)	1 (4.8)		
	N	3 (60)	14 (87.5)	17 (81)		
Age (year)	Mean \pm SD	9.4 \pm 2.3	10.06 \pm 3.06	9.9 \pm 2.86	0.6	-
	Median (Min-Max)	9 (7-13)	10 (6-16)	10 (6-16)		
Sweat Test	<45	4 (80)	4 (25)	8 (38.1)	0.04	12 (1.01-141.33)
	45-75	1 (20)	12 (75)	13 (61.9)		
	>75	0 (0)	0 (0)	0 (0)		
FEV1	<80	4 (80)	6 (37.5)	10 (47.6)	0.1	6.66 (0.59-74.5)
	>80	1 (20)	10 (62.5)	11 (52.4)		

N: Negative; P: Positive; FEV1: Forced Expiratory Volume in 1 Second.

4- DISCUSSION

In this cross-sectional study, 21 children with idiopathic DB and either a normal or borderline sweat test, referred to the Specialized Pediatric Respiratory Clinic of Tabriz University of Medical Sciences during one year, were examined. Overall, five clinically significant CFTR-related gene variants were identified (group 1). Other patients either had only CFTR related polymorphisms or no CFTR related variants (group 2). Comparing the two groups of the patients, the sweat test was significantly higher in group 2. Most patients in group 1 were girls and had a family history of CF. Furthermore, age and FEV1 were lower in group 1 than in group 2. Due to the higher level of sweat test in group 2 compared to group 1, the possibility of recurrent sinopulmonary infections is increased. The lack of electrolyte balance in the mucosa of the respiratory tract is the most common cause of infection (12, 16).

In a study by Milisevic *et al.* on 48 children with idiopathic bronchiectasis in 2013, researchers aimed to determine the association between different CFTR mutations and disease progression. However, all known mutations associated with CF in children were ruled out, and none of the children were diagnosed with CF. In the recent study, the children presented symptoms similar to those observed in our patients (i.e. cough and sputum production). In addition, the FEV1 of children in the study of Milisevic *et al.* was abnormal, and the mean value of sweat test was in the normal range. One case was also identified with the F508 del mutation in the recent report (18). The results of our study are consistent with the findings of this study, highlighting the role of sporadic mutations of G542X and F508 del in the development of bronchiectasis without the involvement of other organs. In another study in 2006, Negimam *et al.* identified CFTR gene mutations in Asian

(Chinese and Singaporean) patients suffering from severe asthma and idiopathic bronchiectasis and reported a higher incidence of CFTR missense mutations including I556V in patients than controls. This indicates that these mutations may increase the risk of idiopathic bronchiectasis (19). In the present study, the 1556V variant was also observed in association with cough, and abnormal sweat test and FEV1. In another study conducted by Watson *et al.* at the American College of Medical Genetics in 2004, people who were willing to participate in a CF screening program were examined for 23 different mutations.

One of the identified mutations was the R117H which is observed in 0.03% of Caucasians and can be associated with a wide range of clinical presentations from CBAVD to classical CF. In the recent study, patients with this genetic variant demonstrated symptoms such as cough, crackle, and sputum production along with normal sweat test and FEV1 (20). In a study by Milosevic *et al.* on 48 Serbian children (19 boys and 29 girls) with idiopathic bronchiectasis, the potential role of CFTR gene variants in the etiology of non-CF dependent bronchiectasis was investigated. Similar to the present report, most of the patients in the recent study were females and heterozygotes for the c.1210-11T> G variant. Patients with this variant showed borderline sweat test results and normal FEV1 (18).

In the study of King *et al.* in 2007, they investigated microbiological profiles of patients with bronchiectasis using sputum culture and reported that the most observed microorganism was Gram-negative *P. aeruginosa* while Gram-positive bacteria including *Staphylococcus aureus* and *Streptococcus pneumoniae* were less common (21). In another study by Shah *et al.*, on patients with bronchiectasis, mutations such as F508 and G542 were identified. These patients had normal

sweat test results and abnormal FEV1, and *S. aureus* and *P. aeruginosa* bacteria were observed in their sputum samples (22).

4-1. Study Limitations

The limitation of this study included: 1) a small sample size, and 2) no comparison with the control group.

5- CONCLUSION

Based on the results of this study, it can be concluded that mutations of the *CFTR* gene in single or heterozygote form can affect the incidence of DB, suggesting that *CFTR* function may be impaired even in those who have normal sweat test results and a healthy copy of the *CFTR* gene. So, it is necessary to differentiate whether DB is a part of *CFTR*-RD failing to meet the diagnostic criteria of CF or a disease independent of CF. It is also recommended to conduct further studies with larger sample sizes to investigate the role of *CFTR* gene variants in different populations and confirm the observations of this study. It is also advisable to consider more genetic determinants such as epigenetic factors in analyzing the role of the *CFTR* gene in DB pathogenesis.

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7- CONFLICT OF INTEREST: None.

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