Iranian Journal of Microbiology

Volume 15 Number 6 (December 2023) 759-764

ORIGINAL ARTICLE

Association between sputum culture results and pulmonary changes in children with cystic fibrosis

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Received: August 2023**, Accepted:** November 2023

ABSTRACT

Background and Objectives: Despite the significant improvement in the prognosis of cystic fibrosis (CF), it is still regarded as the most common life-shortening genetic disease in Caucasian populations. This disease is the most important cause of chronic lung disease and exocrine pancreatic insufficiency in infancy and childhood. The aim of our study was to assess the potential association between bacterial colonization detected by sputum cultures and pulmonary structural and functional changes in Iranian children with CF.

Materials and Methods: In this cross-sectional study, 76 CF children ≥6 years old registered in the CF Foundation of Children's Medical Center Hospital, Tehran, Iran, who underwent high resolution CT scan (HRCT), pulmonary function test, and sputum cultures within a month of each other during the study period were included. For each patient, demographic characteristics (age and sex), results of sputum cultures, forced expiratory volume in 1st second (FEV1), and chest HRCT findings based on the Bhalla scoring system were recorded in a check list.

Results: Sixty seven percent of the patients had positive sputum cultures, with the most commonly isolated microorganism being *Pseudomonas aeruginosa* (mucoid strain). Based on categorization of Bhalla scores, none of the patients had severe pulmonary involvement. FEV1 was mainly >70%. There was a statistically significant correlation between colonization with mucoid *P. aeruginosa* and lower Bhalla scores in children aged 14-16 years (P=0.001). Colonization with mucoid *P. aeruginosa* was also significantly associated with patient's age (P=0.020) and FEV-1 (P=0.001).

Conclusion: Severity of lung involvement in CF children is clearly dependent to mucoid *P. aeruginosa* colonization in airways and this notorious bacterium is the most prevalent one in Iranian CF children. Prompt identification and eradication by proper nebulized and systemic antibiotics can have valueless effects on patients' quality of life and prevent lifelong destructive complications such as bronchiectasis. Timely lung CT scan wisely advised by expert CF treatment team can meticulously detect injuries and it seems to act more efficacious than -still helpful- clinical scores and pulmonary function tests.

Keywords: Cystic fibrosis; Children; Bhalla score; Sputum culture; Forced expiratory volume in 1st second

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INTRODUCTION

Cystic fibrosis (CF) is a monogenic, autosomal recessive disease affecting at least 100 000 people throughout the world, with about 1000 cases being newly diagnosed every year (1, 2).

The highest incidence rate of CF has been reported in the Caucasians with Northern European ancestry, reaching 1 in 2000 to 3000 live births (2).

CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, coding for the CFTR protein, located at the apical surface of epithelial cells, which primarily regulates the transport of chloride and bicarbonate across cell membranes. Absent or dysfunctional CFTR leads to decreased secretion of chloride and increased reabsorption of sodium and water across epithelial cells, subsequently resulting in impaired hydration and clearance of mucus in the organs such as lungs, pancreas, gastrointestinal tract, liver, sweat glands, and reproductive tract (1-3).

The most common mutation of CFTR gene is F508del (i.e. deletion of phenylalanine at codon 508), which is associated with severe clinical manifestations in patients homozygous for the F508del genotype (2). However, Over 2000 mutations of the CFTR gene with varying effects on CFTR function and thus, different phenotypes of the disease have been reported (3).

CF-related tissue abnormalities at the molecular, cellular, tissue, and organ levels can be even detected before birth and that newborns with CF can present with serious complications such as pancreatic insufficiency and meconium ileus (4, 5). Throughout infancy, childhood, and adulthood, other CF-related morbidities such as recurrent respiratory infections, failure to thrive, chronic sinusitis, and male infertility are usually observed (6).

During recent decades, the predicted survival of CF patients has dramatically increased due to advents in symptomatic and prophylactic treatment strategies (4). Based on the United Kingdom CF Registry data from 2011 to 2015, the median survival ages at birth in F508del homozygotes males and females were 46 and 41 years, respectively (7). However, CF is still characterized by premature death, with progressive pulmonary insufficiency brought on by chronic bacterial infections and airway inflammation being the predominant cause of mortality (8).

In children with CF, early defects in mucociliary clearance lead to airway colonization with various bacterial pathogens (e.g. *Staphylococcus aureus* and *Haemophilus influenzae*). As the disease progresses, typical CF pathogens (e.g. *Burkholderia cepacia*, *Achromobacter xylosoxidans*, and in particular, *Pseudomonas aeruginosa*) colonize the airway. Bacterial colonization induces an exaggerated inflammatory response characterized by accumulation and activation of neutrophils, releasing neutrophil elastase that is potentially associated with pathological effects, including destruction of airway tissues, excessive mucus production, impaired mucociliary clearance, and opsonophagocytosis defects (9, 10). This vicious cycle of infection and inflammation finally leads to irreversible structural damage, bronchiectasis, and pulmonary insufficiency (9). Thus, routine monitoring of the structural and functional changes of the lungs and identification of lower respiratory tract pathogens are essential in the management of CF patients.

According to the latest National Institute for Health and Care Excellence (NICE) guideline for CF diagnosis and management, a routine review on the clinical condition of children with clinical evidence of CF-related lung disease, including taking respiratory secretion samples and pulmonary function tests (PFTs), should be performed at least every 8 weeks and chest imagings are recommended to be performed on an annual basis (11). However, using respiratory cultures for direct clinical management of CF is still challenging and studies have reported conflicting results regarding the association between the microbiological yield and structural and functional lung changes in different age groups and study settings (12-15). The aim of our study was to assess the potential association between bacterial colonization detected by sputum cultures and pulmonary structural and functional changes in Iranian children with CF.

MATERIALS AND METHODS

In this cross-sectional study, children who were registered in the Cystic Fibrosis Foundation of Children's Medical Center Hospital, affiliated to Tehran University of Medical Sciences, Tehran, Iran, from 2015 to 2018 were evaluated. Diagnosis of CF was made according to the consensus guidelines from the Cystic Fibrosis Foundation (16). Patients ≥6 years

old who underwent high resolution CT scan (HRCT), forced expiratory volume in 1st second (FEV1), and sputum cultures within a month of each other during the study period were included. Routinely, in the follow-up of patients, pharyngeal cultures were performed every three months, and if positive, treatment was performed according to the type of microorganism grown.

The patients participating in this study were often treated with following antibiotics:

Anti-*Staphylococcus*:
Dicloxacillin 25-50 mg/kg/day in 4 dose or
linezolid 20 mg/kg/day in 2 dose or
nafcillin 100-200 mg/kg/day in 4 dose or
vancomycin 40 mg/kg/day in 3-4 dose

The study was approved by local Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.CHMC.REC.1400.250).

For each patient, demographic characteristics (age and sex), results of sputum cultures, FEV1, and chest HRCT findings were recorded in a check list. Sputum specimen was drawn for each patient and had been cultured in the standard culture medium for bacteria. The Polymerase Chain Reaction PCR -16S sequencing was performed for precise species identification. All HRCTs were performed by an expert radiologist who was blinded to the children's clinical condition and sputum culture and FEV1 results. HRCT findings were evaluated by the same radiologist based on the Bhalla scoring system, assigning scores from 0-3 to each of the following:

Severity of bronchiectasis, peribronchial thickening, extent of bronchiectasis, extent of mucus plugging, sacculations, generations of bronchi involved, and number of bullae. Scores from 0-2 were assigned to emphysema and collapse/consolidation. The total score was calculated by adding individual scores for each item, ranging from 0 to 25 (17). The total Bhalla score was subsequently categorized as follows: excellent (21-25), good (16-20), mild (11-15), moderate (6-10), and severe (0-5).

The statistical analysis was performed using the SPSS® software version 21 (SPSS Inc., Chicago, IL, USA). Descriptive data were reported as mean ± standard deviation or number (percentage). Normally-distributed quantitative variables were compared using the Student's t-test. Non-normally distributed variables were compared using the Mann-Whitney U test. Proportions were compared using the Chi-square Test or Fisher Exact Test, as appropriate. P-values less than 0.05 were considered as statistically significant.

RESULTS

A total of 76 CF patients aged 6-16 years (59% male, mean age 10.4 ± 3.1 years) were enrolled. Half of the patients belonged to the age group of 6-9 years old. Sixty seven percent of the patients had positive sputum cultures, with the most commonly isolated microorganism being *P. aeruginosa* (44.7%). Based on categorization of Bhalla scores, none of the patients had severe pulmonary involvement. FEV1 was mainly >70%. Baseline characteristics of the study population are shown in Table 1.

Table 1. Baseline characteristics of the study population

Characteristics	Frequency
Gender, n (%)	
Male	45 (59.2%)
Female	31 (40.7%)
Age group, n (%)	
6-9 years	38 (50%)
10-12 years	21 (27.7%)
13-16 years	17 (22.3%)
Sputum culture, n (%)	
Negative	25 (33%)
Staphylococcus aureus	17 (22%)
Non-mucoid *Pseudomonas aeruginosa*	22 (29%)
Mucoid *Pseudomonas aeruginosa*	12 (16%)
Pulmonary involvement based on HRCT, n (%)	
Severe	0 (0%)
Moderate	2 (2.6%)
Mild	10 (13.2%)
Good	29 (38.2%)
Very Good	35 (46.1%)
Forced expiratory volume in 1st second (FEV 1)	
<30%	2 (2.6%)
30-70%	24 (31.6%)
>70%	50 (65.8%)

As demonstrated in Table 2, there was a statistically significant correlation between sputum bacterial colonization and Bhalla score categories, with colonization with mucoid *P. aeruginosa* being associated with lower Bhalla scores (P=0.001). When stratified by age, however, the correlation was found to be significant only in 14-16 years age group. Colonization with mucoid *P. aeruginosa* was also significantly associat-

ed with patient's age (P=0.020) and FEV-1 (P=0.001). The frequency of mucoid *P. aeruginosa* positive cultures increased with age, being mainly seen in those aged 14-16 years (Table 3). Moreover, colonization with mucoid *P. aeruginosa* was significantly with decreased FEV-1 (Table 4).

DISCUSSION

Despite the significant improvement in CF prognosis, it is still regarded as the most common life-shortening genetic disease in Caucasian populations (2, 18). In CF patients, altered sputum microstructure and pulmonary ciliary dyskinesia result in mucous plaques formation, providing a microaerobic or even anaerobic setting that contributes to chronic bacterial colonization, among which *P. aeruginosa* is known as the most predominant cause of chronic lung infections (19). Primary identification of *P. aeruginosa* infection is limited by sampling and culturing techniques; however, sputum culture has been widely used as a non-invasive alternative to BAL in expectorating children, providing valuable information regarding lung microbiome and disease state (20).

In our study, the most common pathogens isolated from patients' sputum were the non-mucoid strains of *P. aeruginosa*, followed by *S. aureus.* Similar findings were reported by Khanbabaee et al. and Khodadad et al. suggesting a high rate of *Pseudomonas* infection in Iranian pediatric CF patients (21, 22). On the other hand, according to Montagna et al. *S. aureus* was the main isolated bacterium in CF patients from Southern Italy which might be due to uncommon CFTR mutations leading to a specific local epidemiology (23). Mucoid strains of *P. aeruginosa*

Table 2. Association between sputum microbial culture and Bhalla score categorization

Colonization, n (%)	Bhalla score categorization, n (%)				Total	P-Value
	Moderate	Mild	Good	Very Good		
No growth	0 (0%)	0 (0%)	10 (34.5%)	15 (42.8%)	25 (100%)	
Staphylococcus aureus	1 (50%)	1 (10%)	5 (17.2%)	10 (28.6%)	17 (100%)	
Non-Mucoid *Pseudomonas aeruginosa*	0 (0%)	3 (30%)	12 (41.4%)	7 (20%)	22 (100%)	0.001
Mucoid *Pseudomonas aeruginosa*	1 (50%)	6 (60%)	2 (6.9%)	3 (8.6%)	12 (100%)	
Total	2 (100%)	10 (100%)	29 (100%)	35 (100%)	76 (100%)	

Table 3. Association between sputum microbial culture and age

Colonization, n (%)	Age group, n (%)			Total	P-Value
	6-9	10-13	14-16		
Negative	17 (68%)	6 (24%)	2 (8%)	25 (100%)	
Staphylococcus aureus	9 (53%)	5 (29%)	3 (18%)	17 (100%)	
Non-Mucoid *Pseudomonas aeruginosa*	10 (46%)	7 (32%)	5 (23%)	22 (100%)	0.02
Mucoid *Pseudomonas aeruginosa*	2 (17%)	3 (25%)	7 (58%)	12 (100%)	
Total	38 (100%)	21 (100%)	17 (100%)	76 (100%)	

Table 4. Association between sputum microbial culture and FEV-1

Colonization, n (%)	FEV-1, (%)			Total	P-Value
	<30	30-70	>70		
Negative	0 (0%)	4 (16%)	21 (84%)	25 (100%)	
Staphylococcus aureus	0 (0%)	4 (23.5%)	13 (76.5%)	17 (100%)	
Non-Mucoid *Pseudomonas aeruginosa*	0 (0%)	8 (36.4%)	14 (63.6%)	22 (100%)	0.001
Mucoid *Pseudomonas aeruginosa*	2 (16.7%)	8 (66.6%)	2 (16.7%)	12 (100%)	
Total	2 (100%)	24 (100%)	50 (100%)	76 (100%)	

were most commonly isolated in children aged 14-16 years (Table 3), which is consistent with the development of mucoid morphotypes over time.

HRCT is reported to be the 'gold standard' method for assessment of bronchiectasis in CF patients (24). Several scoring systems have been developed to establish the extent and severity of pulmonary structural changes. We used Bhalla scoring system as it has been found to be superior in pediatric CF patients (25, 26). In the present study, there was a significant association between positive results of sputum culture and Bhalla scoring of HRCT findings in children aged 14-16 years ($P<0.05$). In accordance with our results, Sasihuseyinoglu et al. and Robinson et al. reported significant relationship between the Bhalla score and bacterial growth in the sputum cultures of children with CF (27, 28). On the other hand, Thomas et al. didn't find any significant difference between colonization with *P. aeruginosa* and *S. aureus* regarding lung structural changes detected by HRCT, which might be attributed to the relatively small study population and using a different HRCT scoring system (15). Our findings suggest that early treatment and eradication of nonmucoid *P. aeruginosa* before mucoid transformation can prevent the progression of structural pulmonary changes in CF pediatric patients. To clarify the effect of antimicrobial prevention and treatment on structural lung changes, prospective studies including infants with CF are required.

Airway infection with *P. aeruginosa* has been found to be independently associated with worse outcomes in bronchiectasis; however, whether *P. aeruginosa* is related to a decline in lung function or is only a marker of severity is still a matter of debate (29). It is known that pulmonary function, classically expressed as %FEV1, is a major determinant of treatment strategies and a predictor of survival in CF patients (30). According to our results, colonization with mucoid *P. aeruginosa* was significantly associated with lower FEV1s; as reported by other studies (31, 32). On the other hand, Davies et al. reported that *P. aeruginosa* was associated only with poorer lung function and not with decreased FEV1(33).

One of the limitations of this study is the small sample size and the study's cross-sectional design. A prospective and larger study is needed to confirm these results and would indeed help in further understanding CF progression and disease management. Another limitation of the study is the relatively small number of different clinical parameters measured. For instance, the number of exacerbations and quality of life were not evaluated in this study. Future studies are needed to assess the relationships between these variables.

CONCLUSION

Severity of lung involvement in CF children is clearly dependent to mucoid *Pseudomonas aeruginosa* colonization in airways and this notorious bacterium is the most prevalent one in Iranian CF children. Prompt identification and eradication by proper nebulized and systemic antibiotics can have valueless effects on patients' quality of life and prevent lifelong destructive complications such as bronchiectasis. Timely lung CT scan wisely advised by expert CF treatment team can meticulously detect injuries and it seems to act more efficacious than -still helpful-clinical scores and pulmonary function tests.

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