J Compr Ped.2012;3(1):25-28. DOI: 10.17795/compreped-6944

Comprehensive Pediatrics

www.ComprPed.com

Microbial Colonization and Drug Resistance in Patients with Cystic Fibrosis

Soheila Khalilzadeh [1], Mohammad Reza Bolursaz [1], Nooshin Baghaie [1], Elaheh Heydarian Fard [1], Maryam Hassanzad [1*], Habib Emami [2]

[1] Pediatric Respiratory Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

[2] Tobacco Prevention and Control Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 20 Sep 2011
Revised: 30 Sep 2011
Accepted: 10 Oct 2011

Keywords:
Cystic Fibrosis
Child
Pseudomonas Aeruginosa
Drug Resistance

ABSTRACT

Background: Cystic fibrosis (CF) is a genetic disease with an autosomal recessive pattern of inheritance. CF caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and characterized by impaired transport of chloride ions across the cell membrane. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* have been identified in the cultures of respiratory secretions of CF patients, and infections of these microorganisms are associated with high rates of morbidity and mortality. In patients with CF, severe inflammation of the airway can cause advanced bronchiectasis, which may result in respiratory failure and death.

Objectives: This study aimed at evaluating the clinical findings of laboratory tests, bacterial colonization, and drug resistance in children and young adults with CF who hospitalized at the Pediatric Pulmonary Department of Masih Daneshvari Hospital, Tehran, Iran.

Patients and Methods: This cross-sectional study was conducted on 22 children and young adults with CF who were hospitalized at the Pediatric Department of Masih Daneshvari Hospital between 2006 and 2011, with convenient sampling. All analysis performed with SPSS V 11.5 and *P* values less than 0.05 considered as statistically significant.

Results: A total number of 23 patients evaluated, including 12 (52.2%) girls and 11 (47.8%) boys. Patients had a mean age of 14.5 ± 6.7 years. The sputum cultures of 10 (43.5%) (95% CI, 23.2-63.7%) patients [5 female (41.7%) and 5 male (45.5%)] were positive for *P. aeruginosa* and those of 2 (8.7%) (95% CI, 0-20.2%) were positive for *S. aureus* [1 female (8.3%) and 1 male (9.1%)]. The sputum cultures of 2 (8.7%) (95% CI, 0-20.2%) other patients were positive for nontuberculous mycobacteria (NTM). The purified protein derivative (PPD) skin test yielded negative results in 10 male (46%) and 12 female (55%). Based on the antibiograms obtained from sputum cultures, we found that *P. aeruginosa* had the highest susceptibility to ciprofloxacin (71.4%), followed by amikacin (50%), ceftazidime (30%), and ceftriaxone (18%). In this study, the annual prevalence of respiratory infections in patients who given nebulized antibiotic prophylaxis was significantly lower than that in patients who did not receive this treatment ($P < 0.05$).

Conclusions: Our study results (43.5% cultures positive for *P. aeruginosa* and high resistance to antipseudomonal drugs) suggest that the use of inhaled medications for prophylaxis in CF patients could result in a decreased rate of hospitalization and reduction in CF-related complications.

* *Corresponding author:* Maryam Hassanzad, Pediatric Respiratory Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran. Tel: +98-2127122458, Fax: +98-2126109484, *E-mail:* p_nritld@yahoo.com

DOI: 10.17795/compreped-6944

Implication for health policy/practice/research/medical education:
Exacerbations of CF is important in quality of life in these patients, we must choose the best and the most effective antibiotic for their treatment. Therefore, we worked in this field and we determined the responsible microbes and their antibiogram.

Please cite this paper as:
Khalilzadeh S, Bolursaz MR, Baghaie N, Heydarian Fard E, Hassanzad M, Emami H. Microbial Colonization and Drug Resistance in Patients With Cystic Fibrosis. *J Compr Ped*. 2012;**3**(1):25-8. DOI: 10.17795/compreped-6944

1. Background

Cystic fibrosis (CF) is the most prevalent autosomal recessive disease in Caucasians (1); however, no data regarding the prevalence of CF in Iran is available. Although the genetic defect responsible for the development of CF is still unknown, the association between abnormal cystic fibrosis transmembrane conductance regulator (CFTR) protein and chronic pulmonary diseases is becoming more apparent (2).

Chronic respiratory infections that may occur at the early stages of life are among the main symptoms of CF. Pulmonary infections are the most common cause of morbidity and mortality in children with CF and result in early death of 90% of CF patients (2). In studies conducted in the last 3 decades, *Pseudomonas aeruginosa* was the most frequent microbial agent detected in the airways of CF patients. Bacterial infections are the most common cause of morbidity and mortality in this group of patients (1). Various microorganisms comprise the microbial flora of the airways, the most important of which are *Staphylococcus aureus*, *P. aeruginosa*, and *Haemophilus influenza*. The percentage of colonization of the aforementioned microorganisms in children aged 6–10 years was 60%, 40%, and 25%, respectively (1). In recent years, the incidence and prevalence of infections with methicillin-resistant *S. aureus* (MRSA), *Stenotrophomonas*, *Burkholderia cepacia*, *Alcaligenes xylosoxidans*, *Klebsiella* species, and nontuberculous mycobacterium (NTM) have increased (3). Among these pathogens, *B. cepacia* is especially important because it can cause severe respiratory failure and eventual death (4). Moreover, these bacteria can spread among patients and cause epidemics in CF centers (5). The prevalence of *B. cepacia* in CF patients in Germany in the year 2000 was 2.4% (6). Additionally, the percentage of colonized patients increases with age and severity of disease. The results of previous studies suggest that antibiotic prophylaxis in patients with respiratory infections can increase forced expiratory volume in 1 second (FEV1) values and decrease the duration of hospital stay (7, 8). Researchers have also shown that maintenance therapy with antipseudomonal antibiotics in children with CF can improve pulmonary function test (9).

2. Objectives

This study aimed at evaluating the clinical findings of laboratory tests, bacterial colonization, and antibiotic resistance in children with CF who hospitalized at the Pediatric Pulmonary Department of Masih Daneshvari Hospital.

3. Patients and Methods

This cross-sectional study was conducted using the medical records of 23 patients with CF who were hospitalized at the Pediatric Department of Masih Daneshvari Hospital in Tehran, Iran, between 2006 and 2010. All patients with a positive sweat test (chloride level > 60 mg/L) and clinical symptoms indicating CF were included in the study as a convenient sampling manner. Information regarding age, sex, family history of CF, consanguinity of the parents, and laboratory test results (including sputum culture and microbial antibiotic susceptibility/resistance test results) collected from the patients' medical records. Sputum culture was performed on MacConkey agar, chocolate media, and blood agar. Next, the disc diffusion method was used for detection of microbial antibiotic susceptibility. Antibiograms were obtained using cotrimoxazole, ceftriaxone, ceftazidime, amikacin, and ciprofloxacin kits (Darvash Company and Padtanteb Company) (these kits were selected because of the limited variety of kits available in the referral laboratory). Data were analyzed using SPSS version 11.5. Prophylactic treatment was administered to all patients after discharge from the hospital. ANOVA and *t* tests were used to evaluate the relation between variables; normality of numeric variables in different states of categorical variables was assessed with K-S test. *P* values less than 0.05 were considered statistically significant.

4. Results

The 23 CF patients included 12 female (52.2%) and 11 male (47.8%). The mean age of the patients was 14.5 ± 6.7 years. The mean age of the patients at the time of diagnosis was 9.5 ± 7.3 years. A family history of CF was found in 1 (4.3%) patient. Parental consanguinity was noted in 16 (69.6%) (95% CI, 50.8-88.4%) of the cases. In 18 (78.3%) (95% CI, 61.4-95.1%) of the patients, growth indices were below 10%. In 3 (13%) (95% CI,0-26.8%), these figures were below 3%. Evaluations showed that the most common complaints were productive cough, growth retardation, and gastrointestinal complications (*Figure 1*).

Six-minute walk tests yielded abnormal results in 22 subjects. Pulmonary function tests showed a severe restrictive pattern in 8 (34.7%) patients and a severe obstructive pattern in 3 (13%) patients. Of the 23 patients, 10 (5 male

and 5 female patients) had a positive sputum culture for *P. aeruginosa* and 2 (1 male and 1 female patient) had a positive sputum culture for *S. aureus*. Positive sputum culture for NTM was observed in 2 patients. TST (tuberculin skin test) yielded negative results in 12 female (55%) and 10 male (46%) patients. In the present study, the highest susceptibility levels were recorded for ciprofloxacin (71.41%), amikacin (50%), ceftazidime (30%), and ceftriaxone (18%) (*Figure 2*). Hepatic cirrhosis, cor pulmonale, and acute appendicitis (which necessitated appendectomy) were detected in 2 (6%), 4 (18%), and 2 (6%) cases, respectively. One patient had a history of pulmonary tuberculosis (3%). After diagnosis of CF, prophylaxis with nebulized antibiotics, chest physiotherapy, and supplementary vitamins were prescribed for all patients. According to the medical records of the patients, 13 patients received regular follow-up, whereas 9 patients did not. Statistical comparison between the 2 groups (patients who underwent follow-up and those who did not) revealed a significant decrease in the number of respiratory infections per year in those who were receiving regular prophylaxis ($P < 0.05$). Chest CT scan findings are summarized in *Figure 3*.

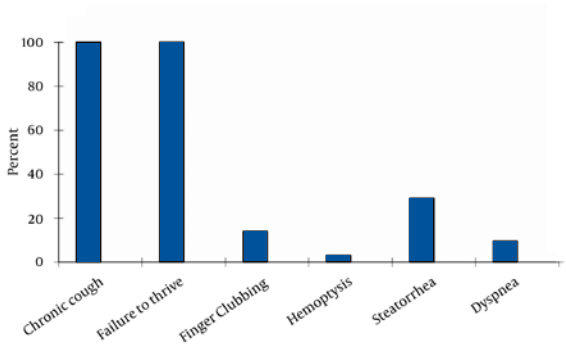

Figure 1. Distribution of Clinical Symptoms in CF Patients

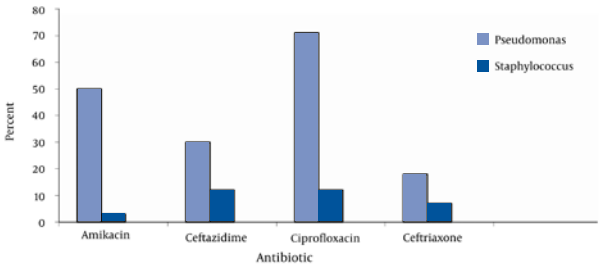

Figure 2. Antibiotic Susceptibility of Different Bacterial Species

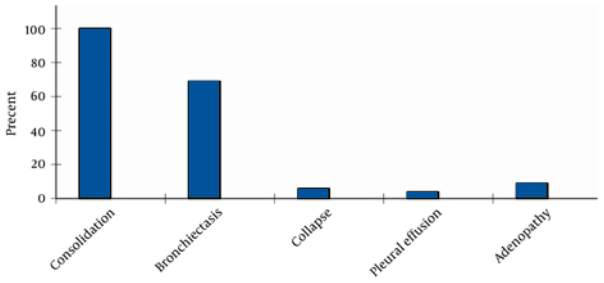

Figure 3. Chest CT Scan Findings in CF Patients

5. Discussion

The lungs of CF patients are colonized with various microorganisms. In these patients, airway inflammation can start at an early age. Epidemiological studies have not been able to elucidate the routes of transmission and prevalence of cross-infections. At present, the main clinical problem in CF patients is airway infection with *P. aeruginosa* and associated inflammation. In this study, the main pathogen in patients with chronic pulmonary infections was *P. aeruginosa*. Although treatment and antibiotic prophylaxis have decreased morbidity and mortality in CF patients, the problem of resistance to antibiotics for *P. aeruginosa* still exists (9-12). Despite treatment, *P. aeruginosa* can remain in the lungs of CF patients and lead to serious problems.

The role of inflammation in destroying tissue and the resultant dysfunction and failure of the organ has been shown in a number of studies (13). Use of anti-inflammatory treatment in infectious patients can result in clinical recovery or improvement (14, 15). Various studies have shown that inflammation and bacterial infection occur at an early age, before the manifestation of the main clinical symptoms. The results obtained by Flume *et al*. for the clinical symptoms of CF (13) are in agreement with those presented here. Bronchiectasis causes focal hemorrhagic pneumonia, which can explain the occurrence of bloody sputum in CF patients (14). Chest CT scan findings show that 48% of patients develop bronchiectasis (15). In fact, CF is among the main causes of bronchiectasis. In a study performed in 1998, Coming *et al.* showed that in 39% of bronchiectatic patients, CF was the main cause (15). In CF patients, accumulation of purulent mucous secretions in the airways and destruction of lung parenchyma results in the development of cor pulmonale (16). This condition was noted in 3% of the cases assessed in this study.

One of the important complications of CF is the development of recurrent respiratory infections. Administration of antibiotic prophylaxis is an effective method for decreasing the prevalence of pneumonia in CF patients (17). The findings presented here show that proper antibiotic prophylaxis could decrease the prevalence of pneumonia and reduce the number of annual respiratory infections in CF patients. In a study conducted in the US on 520 CF patients, administration of tobramycin significantly decreased the hospitalization rate (18). Another study showed that use of inhaled tobramycin (2-3 times per day) in CF patients was associated with improved pulmonary function and decreased number of PA (*P. aeroginosa*) in the sputum of these patients (19).

The presence of NTM in CF patients is being increasingly recognized. This may be due to high environmental exposure to NTM and the rapid progression of the disease.

In this study, 2 of the 23 patients had positive cultures for NTM (MAC) (mycobacterium avium complex). We recommend that in patients who are not responding to the usual CF treatment, the presence of NTM should be evaluated (20).

It is generally believed that long-term use of oral or inhaled antibiotics can suppress respiratory infections (21). In Iran, patients mostly use nebulized gentamycin or amikacin with azithromycin every other month because the cost of inhaled tobramycin is high. This method although decreases the number of microorganisms, can be associated with drug-induced complications or development of drug resistance. The results of this and other studies suggest that proper administration of prophylactic antibiotics (especially in children with positive culture for *P. aeruginosa*) along with inhaled medications, vitamins, and chest physiotherapy may decrease the disease prevalence and reduce the rate of complications and frequency of hospital stays in children with CF (22). In a study conducted by Anthony and colleagues in 2002 on 140 children with CF, *H. influenza* and *B. cepacia* were found to be the most prevalent colonizing microorganisms in the airways (75% with positive culture). Their results are comparable with the results presented here, which showed that 43% and 8.6% of cultures in this study were positive for *P. aeruginosa* and *S. aureus*, respectively (23). In a multi-center study conducted by Eftekhar and coworkers on 64 CF patients in Tehran, *P. aeruginosa* was reported in 21 cases (6 cases of mucoid and 15 cases of classic) (24). In the current study, the highest antibiotic susceptibly was to ciprofloxacin (71.41%), amikacin (50%), ceftazidime (30%), and ceftriaxone (18%).

Considering all the above factors, it can be concluded that the lungs in CF patients are ideal environment for the growth of pathogenic microorganisms. As the number of drug-resistant colonies of pathogens increases, treatment of infections in CF patients becomes more challenging. However, use of vaccines and inhaled antibiotics as prophylactic agents could be beneficial in this respect.

Acknowledgments

None declared.

Financial Disclosure

None declared.

Funding/Support

None declared.

Author's Contribution

None declared.

References

1. Behrman RE, Kliegman R, Jenson HB. Cystic Fibrosis. In: Thomas F, editor. *Nelson textbook of pediatrics*. 17th ed. Philadelphia: Saunders Elsevier; 2004.
2. Kabra SK, Kabra M, Shastri S, Lodha R. Diagnosing and managing cystic fibrosis in the developing world. *Paediatr Respir Rev*. 2006;**7**(Suppl 1):S147-50.
3. Fares F, David M, Lerner A, Diukman R, Lerer I, Abeliovich D, et al. Paternal isodisomy of chromosome 7 with cystic fibrosis and overgrowth. *Am J Med Genet A*. 2006;**140**(16):1785-8.
4. Marks JH. Airway clearance devices in cystic fibrosis. *Paediatr Respir Rev*. 2007;**8**(1):17-23.
5. Fields TM, Michel SJ, Butler CL, Kriss VM, Albers SL. Abdominal manifestations of cystic fibrosis in older children and adults. *AJR Am J Roentgenol*. 2006;**187**(5):1199-203.
6. Green A, Kirk J. Guidelines for the performance of the sweat test for the diagnosis of cystic fibrosis. *Ann Clin Biochem*. 2007;**44**(Pt 1):25-34.
7. Wall M. On staphylococcal prophylaxis in CF. *Pediatr Pulmonol*. 2007;**42**(2):186; author reply 7.
8. Doron S, Gorbach SL. Probiotics: their role in the treatment and prevention of disease. *Expert Rev Anti Infect Ther*. 2006;**4**(2):261-75.
9. Kulczycki LL. Five decades of cystic fibrosis (1938-1988). *Acta Univ Carol Med (Praha)*. 1990;**36**(1-4):7-12.
10. Therrell BL, Lloyd-Puryear MA, Mann MY. Understanding newborn screening system issues with emphasis on cystic fibrosis screening. *J Pediatr*. 2005;**147**(3 Suppl):S6-10.
11. Katznelson D, Ben-Yishay M. Cystic fibrosis in Israel: clinical and genetic aspects. *Isr J Med Sci*. 1978;**14**(2):204-11.
12. Santana MA, Matos E, do Socorro Fontoura M, Franco R, Barreto D, Lemos AC. Prevalence of pathogens in cystic fibrosis patients in Bahia, Brazil. *Braz J Infect Dis*. 2003;**7**(1):69-72.
13. Merqury N MJ. Clinical aspects of cystic fibrosis. *Med Sci Monit*. 2000;**11**(12):325-8.
14. Flume PA, Yankaskas JR, Ebeling M, Hulsey T, Clark LL. Massive hemoptysis in cystic fibrosis. *Chest*. 2005;**128**(2):729-38.
15. Santamaria F, Grillo G, Guidi G, Rotondo A, Raia V, de Ritis G, et al. Cystic fibrosis: when should high-resolution computed tomography of the chest Be obtained? *Pediatrics*. 1998;**101**(5):908-13.
16. McIlwaine M. Chest physical therapy, breathing techniques and exercise in children with CF. *Paediatr Respir Rev*. 2007;**8**(1):8-16.
17. Harrison F. Microbial ecology of the cystic fibrosis lung. *Microbiology*. 2007;**153**(Pt 4):917-23.
18. Ryan G, Mukhopadhyay S, Singh M. Nebulised anti-pseudomonal antibiotics for cystic fibrosis. *Cochrane Database Syst Rev*. 2003(3):CD001021.
19. Ratjen F, Doring G, Nikolaizik WH. Effect of inhaled tobramycin on early Pseudomonas aeruginosa colonisation in patients with cystic fibrosis. *Lancet*. 2001;**358**(9286):983-4.
20. Ebert DL, Olivier KN. Nontuberculous mycobacteria in the setting of cystic fibrosis. *Clin Chest Med*. 2002;**23**(3):655-63.
21. Adeboyeku D, Scott S, Hodson ME. Open follow-up study of tobramycin nebuliser solution and colistin in patients with cystic fibrosis. *J Cyst Fibros*. 2006;**5**(4):261-3.
22. Hart CA, Winstanley C. Persistent and aggressive bacteria in the lungs of cystic fibrosis children. *Br Med Bull*. 2002;**61**:81-96.
23. Eftekhar F RF, Khodadad A. Evaluation of Pseudomonas aeruginosa colonisation in patients with cystic fibrosis. *Iran J of Infection & Tropical Medicine*. 2003;**20**(8):14-7.
24. Kim DN, Lazarus AA. Management of bronchiectasis. *Dis Mon*. 2008;**54**(8):540-6.