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The Value of Sputum Polymerase Chain Reaction for Detection of Nontuberculous Mycobacteria in Cystic Fibrosis Patients with Negative Nontuberculous Mycobacteria Sputum Culture

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Abstract

Background: Cystic Fibrosis (CF) is a life-threatening autosomal recessive disease. The purpose of this study was to evaluate the value of Polymerase Chain Reaction (PCR) in CF patients with Nontuberculous Mycobacteria (NTM) negative sputum culture.

Methods: This is a descriptive cross-sectional study. The population included all children with CF, aged between 5 - 18 years old, with an NTM negative sputum culture. The patient's sputum samples were sent for smear and culture of NTM, RFLP PCR, and PCR sequence.

Results: In total, 57 CF patients with negative NTM sputum culture were enrolled. Nine patients (15.78%) had positive sputum PCR for NTM. Among these strains, Mycobacterium simiae was the most common one with 5 cases (8.77% of total positive cases).

Conclusion: PCR can be used as an alternative diagnostic method for NTM in CF patients with negative NTM sputum culture, always under clinical suspicion of the disease.

Keywords: Cystic Fibrosis, Nontuberculous Mycobacteria, Polymerase Chain Reaction, Sputum

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Introduction

Cystic Fibrosis (CF) is a life-threatening autosomal recessive disease caused by mutations in the gene that encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein. This gene is located on chromosome 7. This mutation leads to involvement of the pulmonary and gastrointestinal systems and complications in other organs. This genetic defect leads to the accumulation of thick mucosa in the patient's airway, which provides the medium for the development of infections. As a result, respiratory infections are the main problems of these patients (1). Nowadays, with the advancement of treatments and increased life span, the incidence of opportunistic infections resistant to common drugs has gradually increased. One of the opportunistic organisms is Nontuberculous Mycobacteria (NTM). Since 1990, several studies have been published indicating an increase in the detec-

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↑What is "already known" in this topic:
The importance of respiratory infections in patients with cystic fibrosis is well known.
NTM is one of the opportunistic organisms that sometimes cause problems in these patients.
Currently, the standard diagnostic method for these microorganisms is sputum culture.

→What this article adds:
PCR can be used to detect NTM in cystic fibrotic patients who have a negative sputum culture but are still suspected of having NTM infection.

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tion of this organism from the respiratory tract samples in CF patients (2). Numerous studies have been performed on non-tuberculosis Mycobacterium infections in CF patients, and different ranges of NTM species have been reported (3, 4). NTM are usually capable of causing disease in various organs like the skin, soft tissue, lymph nodes and disseminated infections. However, in CF patients, they almost always involve the lungs. These organisms are a large family that in the past were called atypical mycobacteria (4, 5). Accordingly, the U.S.Cystic Fibrosis Foundation and the European Cystic Fibrosis Society (ECFS) recommend that patients with CF should be screened annually for NTM in respiratory samples. The role of culture of respiratory tract samples for this organism detection has been proven and it is routinely used worldwide (4). There are limited studies about the role of PCR in the detection of NTM in CF patients. Therefore, the purpose of this study was to evaluate the value of PCR in CF patients with NTM negative sputum culture.

Methods

Study design

This is a descriptive cross-sectional study. The population included all children with CF, aged between 5 and 18 years old, referred to the CF Center of the Children's Medical Center Hospital, from March 2016 to March 2016, with NTM negative sputum culture. Inclusion criteria were: children aged 5-18 years old with CF confirmed by UCFF criteria and negative sputum culture in terms of NTM. Exclusion criteria included unwillingness to enter the study and inability to repel sputum.

Sample size

The statistical population comprehended all patients with CF with inclusion criteria, referred to the CF Center of Children Medical Center Hospital for one year (2016).

Intervention

All children aged 5 to 18 years with CF with NTM negative sputum culture were evaluated. At first, written consent was obtained from the children and their parents to participate in the study. Individual questionnaires were completed for each patient, including demographic information such as age, gender, and duration of the disease. The patient's sputum samples were collected in the following way: initially, the patient washed his mouth with normal saline then the specimen was collected and delivered to the Hospital's laboratory. The collected samples were transferred in ice to the Pasteur Institute lab within one hour. Part of the samples was sent for smear and culture of NTM and the rest were sent to RFLP PCR and PCR Sequence. The Ziehl Neelsen staining and Levenstein culture medium were used for NTM detection. The Roch diagnosties deutschland GmbH Mannheim Kit (Germany) was used for PCR. The best method for detection of NTM isolates is MLSA (Multilocus sequence analysis). We used this method for detection with three housekeeping genes including hsp65, rpoB, 16S rRNA. Our goal was just to investigate for NTM by other methods (PCR) in cases where sputum culture was negative, and we did not intend to investigate the relationship between the presence of NTM and the presence of disease.

Ethical considerations

All patient information was kept confidential, and informed consent was obtained from all children and their parents. The study was approved by the Ethics Committee of Tehran University of Medical Sciences (ethics number: IR.TUMS.MEDICINE.REC.1395.1725). All costs of the tests were provided by the study executives and the patients did not pay extra. The results of the tests were reported to the patients and the necessary medical treatment was carried out.

Statistical analysis

The results of the tests were recorded in the questionnaires. Finally, the data were analyzed by SPSS statistical software (version 20). Study participant characteristics and frequency of NTM were explored with descriptive statistical methods. The mean ± standard deviation and frequency were used to report the variables. To find the frequency of Mycobacterium strains in patients, descriptive analysis was performed using SPSS software.

Results

Fifty-seven CF patients with negative NTM sputum culture were enrolled. Twenty-four patients (42%) were boys, and 33 (58%) were girls. Their mean age was 12.22 ± 2.44 (range 6 to 17 years). Of these, 5 patients (8.7%) were diagnosed during the current year and 52 children (91.3%) were previously diagnosed. NTM sputum Cultures and smears were negative in all children. Nine patients (15.78%) had positive sputum PCR for NTM; the strains obtained are summarized in Table 1. Among these strains, Mycobacterium simiae was the most common with 5 cases (8.77% of to tal positive cases). All patients with positive PCR were females and were previously known cases of the disease.

Discussion

We found that in cases where there is a strong clinical probability about the role of NTM, PCR can be used for the detection of NTM. NTM is a large family of organisms in the environment that can cause chronic pulmonary infections, especially in people with underlying pulmonary disease, such as CF (6). NTM detection from sputum or other respiratory tract samples in patients with CF has increased in different countries. Reported cases in the United States range from 0% to 28% with an average of 12% (1). Catherine Pierre-Audigier et al. reported an overall incidence of NTM in 385 patients as 8.1% (7).

Table 1. Frequency of mycobacterium strains

Strain type	Number	Percentage
Mycobacterium simiae	5	8.77
Mycobacterium chelonae	3	5.26
Mycobacterium gordonae	1	1.75
Total	9	15.78

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Negative NTM sputum culture is not uncommon in children suspected of NTM infection (8). Consequently, in patients with clinical suspicion of NTM infection but with ~~negative~~ culture, it is necessary to use alternative methods to detect this organism. We evaluated the value of NTM PCR in CF patients with negative NTM sputum culture. Interestingly, the PCR was positive in 15.78% of cases.

Several factors can explain this discrepancy between culture and PCR results. For example, culture overgrowth by non-mycobacterial species can substantially reduce NTM detection, while sample decontamination techniques used to prevent this event can reduce mycobacterial viability. In addition, CF patients often receive antibiotics such as macrolides and quinolones that also reduce the chance of positive sputum culture (9). Gianny p.scoleri et al. studied a BAL sample of CF patients with negative culture but positive PCR. High levels of Hemophilus influenza were detected. These findings suggest that the presence of other non-mycobacterium species may result in the failure to isolate NTM (10). Of course, this should be taken into account. Positive PCR does not necessarily mean that viable bacterial cells are present (11, 12). In our study, Mycobacterium simiae was the most abundant strain obtained by sputum PCR. These findings are similar to the results of the multicenter study of Isaac Levy in Israel. In his study, 40.5% of CF patients had Mycobacterium simiae positive sputum culture (13). Also, Amal Hamihereported that Mycobacterium simiae is a common organism found in patients with underlying pulmonary disease (non-CF patients) (14). It seems that, given the common geographic region (Middle East), particular attention should be paid to this organism. Various results have been obtained from studies conducted in different countries (4). In a multicenter study by Anne Laure Roux and colleagues in France, the overall incidence of NTM in different regions of the country was reported from 3.7% to 9.6% (median 6.6%). Mycobacterium abscessus complex (MABSC) and mycobacterium avium complex were the most common and the second most common organism, respectively. This difference in frequency and type of organism can be due to social or geographical differences, as well as age and antibiotic usage. As in the French study, there were also differences between different regions of a country (13). We found an overall incidence of NTM of 15.78%, which is lower than the reported values in Iceland (40.9%) (15). However, it is closer to the figures reported in the United States (12%) (4). In general, the prevalence of NTM in respiratory specimens of CF patients increases with age. The study by Catherine Pierre-Audigier depicted that the overall incidence of NTM in CF patients under 15 years of age was 4.8% versus 14.9% in patients 15 years of age or older. On the other hand, the organisms obtained at different ages can be different, as, in this study, Mycobacterium avium complex was only detected in patients older than 15 years of age. In a study by Davari M et al., which was conducted in Iran between 2013 and 2015 in order to detect NTM in adults (included CF patients and non-CF patients), mycobacterium fortuitum was the most common strain (63.9%).In this study, of the six CF patients who had positive NTM-culture, 5 cases were mycobacterium fortuitum and one case was Mycobacterium abscessus (5). The statistical difference between this study and ours can be attributed to the age of the evaluated population. As the mean age of our patients was 12.22 ± 2.44 years, compared with 49.6 ± 16.6 in the study of Davari M and colleagues (5). The most important limitation of this study was that the younger children could not expel sputum, so there was no possibility to send samples for testing. Another limitation was the high cost of the PCR. Due to the difficulty of taking sputum samples, only one sample was taken, but in standard conditions, two of the three lung samples must be positive to be considered valuable.

We propose that future studies be multi-centered and with larger sample size. In patients who are not able to dislodge sputum, samples by BAL should be taken. Also, it is necessary to evaluate the association of clinical symptoms and radiological findings with different strains of NTM.

Conclusion

According to this study, PCR can be used as an alternative diagnostic method for NTM in CF patients with negative NTM sputum culture but with clinical suspicion of the disease.

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Ethical approval

The study was approved by the Ethics Committee of Tehran University of Medical Sciences (ethics number: IR.TUMS.MEDICINE.REC.1395.1725).

Conflict of Interests

The authors declare that they have no competing interests.

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