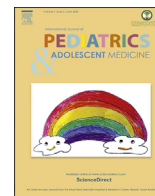


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Original article

The prevalence of viral infections in children with cystic fibrosis in a tertiary care center in Saudi Arabia

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ABSTRACT

Introduction: Studies have shown that pulmonary exacerbations in cystic fibrosis (CF) patients are associated with respiratory viruses. The most common agent causing viral infections in patients with CF before the age of 3 years is respiratory syncytial virus.

Objectives: To obtain the prevalence of the different types of viral infection in CF patients and to identify its relation with the type of bacterial infection, (CFTR) mutations and pulmonary function test (PFT).

Methodology: A retrospective charts review of 387 patients with CF of all age groups who were screened for the detection of viruses during respiratory exacerbation from the period of January 1, 1984 to June 1, 2016.

Results: A total of 159 CF patients had pulmonary exacerbation and had viral PCR obtained. Fifty-eight patients (36%) had positive viral PCR. Males were more commonly infected in 30/58 patients (52%) compared to females in 28 patients (48%). Forty-five of 58 patients (78%) were alive and 13 patients (22%) died. Rhinovirus was the most frequently reported viral PCR in 33/74 sample (45%). Out of 74 viral PCR, 41 (55.4%) were during the colder seasons (October–February) and 33 (44.5%) during the warmer seasons (March–September). During viral infection and viral recurrence, there was an increase in bacterial colonization specifically of *H. influenza* and *staphylococcus aureus*. The most common CFTR mutation for the CF viral infection is: 3120+1G>A in Intron 16 in 11/57 patients (19%). The Eastern Province had the highest viral infection of 24 out of 57 patients (42%). Follow-up PFT post viral infection showed no significant difference in the type and the severity of PFT compared to the initial PFT during the viral illness.

Conclusion: Viral infections contributed to the increase in morbidity and mortality of CF patients in our population, and rhinovirus was the most common causative agent. Viral infections and viral recurrence increased the prevalence of bacterial infection of specific pathogens such as *H. influenza* and *S. aureus*. Physicians should be aware to prevent progressive lung damage in CF patients by treating the concomitant viral and bacterial infections. Viral infection may be associated with some common CFTR mutations.

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1. Introduction

Cystic fibrosis (CF) is a progressive illness that affects many

systems including respiratory, gastrointestinal, urogenital, and sweat glands [1,2]. CF is an inherited autosomal recessive disease caused by mutation of the CF transmembrane conductance regulator (CFTR) gene [1]. One in 2000–3000 live births in Caucasian populations is affected with CF, which makes it the most common autosomal recessive disease [2]. Deletion of phenylalanine in amino acid position 508 (deltaF508) on chromosome 7 is considered as the most common mutation in North America and Western Europe

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Over the past 20 years, the life expectancy of CF patients has improved significantly [2]. Respiratory viruses were reported to cause respiratory damage in patients with cystic fibrosis [3,4].

Wat et al. suggested that respiratory viruses were associated with 40% of the pulmonary exacerbations in CF patients, which led to disease progression and pulmonary function test (PFT) abnormalities [5].

Armstrong et al. showed that 52% of CF infants who were admitted to the hospital because of respiratory symptoms had viral infections. Thirty-five percent of them had acquired *Pseudomonas aeruginosa* (PA) compared with 6% of those who were not hospitalized. The author also showed an association between respiratory viral infections and the inflammatory changes in the airway [6]. This is because respiratory viral infections cause respiratory epithelial injury, which leads to increased adherence of bacterial pathogens to pharyngeal cells. These bacteria include *Staphylococcus aureus* (*S. aureus*), *Hemophilus Influenzae* (*H. influenzae*), *Streptococcus pneumoniae* (*S. pneumoniae*), and *Pseudomonas aeruginosa* (PA) [6].

It has been reported that the most common agents causing viral infections in patients with CF before the age of 3 years is Respiratory Syncytial virus, Influenza A, Parainfluenza 3, and Adenovirus were more commonly detected in patients above the age of 3 years [6].

Hiatt, P. W et al. suggested that CF infants with respiratory viral infection are at a significant risk for lower respiratory tract infections, hospitalization, and long-term deterioration of lung function [7].

Bellinghausen et al. suggested that the pre-exposure to normal respiratory bacteria may modify the response of lung epithelia to viruses and may explain the susceptibility to pulmonary exacerbations [8].

Banjar et al. found that (PA) was detected in 44% of 96 CF patients, while (*H. influenzae*) was detected in 17% CF patients, (*S. aureus*) in 15%, (*S. pneumoniae*) in 6%, and *Methicillin-resistant Staphylococcus aureus* (MRSA) in 4% CF patients. The authors also found that the development of early PA resistance to antibiotics have been shown to contribute to early mortality in CF patients [9].

We carried out our study to determine the prevalence of viruses in cystic fibrosis patients and to find out its correlation with bacterial cultures, CFTR mutations and Pulmonary Function Test (PFT) in a tertiary care center in Saudi Arabia, which is considered the main referral center for CF patients.

1.1. Objectives

To obtain the prevalence of viral infection in CF patients and to identify its relation to bacterial infection, (CFTR) mutations and pulmonary function test (PFT).

2. Methodology

After obtaining the ethical approval, we retrospectively reviewed charts of 387 patients with cystic fibrosis of all age groups who were screened for detection of viruses during respiratory exacerbation from January 1, 1984 to June 1, 2016. Polymerase chain reaction (PCR) of respiratory viruses were extracted and analyzed according to the standard method [10,11].

2.1. Definitions

Patients with CF disease are diagnosed if they have the phenotypic CF characteristics, which include chronic sinopulmonary disease, characteristic gastrointestinal and pancreatic

abnormalities and absence of the vas deferens, and a sweat chloride concentration above 60 mmol/l or loss of CFTR-mediated trans epithelial conductance in the nasal epithelium" [12].

2.1.1. Respiratory exacerbation

It is defined as "a recent change in at least two of the following conditions: increases dyspnea, change in sputum volume or color, increased cough, increased malaise, fatigue or lethargy, anorexia or weight loss, or decrease in pulmonary function by 10% or more/radiographic changes" [13].

Viral recurrence is defined as viral re-infection with similar or different virus after having a negative PCR within a period of 6 months [14].

2.2. Inclusion criteria

All CF patients of all ages who underwent PCR multiplex for respiratory viruses during their follow up from the period January 1, 1984 to June 1, 2016 were included [10,11].

2.3. Type of samples

Induced sputum samples were obtained from patients above 4 years of age. Nasopharyngeal aspirates (NPA) were taken from patients who were unable to expectorate below the age of 4 years. Broncho alveolar lavage (BAL) samples were taken from severely ill patients with pulmonary disease and from those who were admitted to the hospital.

2.4. Method of sample collection

Multiplex PCR test for the detection of respiratory viruses and bacterial cultures were collected and processed according to standard methodology. Samples were collected following standard hospital precautions [9–11].

2.5. Statistical method

The T-Test was used to calculate the continuous variables, median, mean, and standard deviation. All values were expressed in mean \pm standard deviation (SD), and the results were presented at a level of significance of $P < .05$.

2.6. Method used to assess lung capacity

PFT was done according to standard procedure. FEV1 more than 70 is considered mild, between 60 and 69 is moderate, between 50 and 59 is moderately severe, between 35 and 49 is severe, and less than 35 is very severe [15].

3. Results

We examined (NPA/Sputum and BAL) samples from 387 cystic fibrosis patients at respiratory exacerbation in a tertiary care center. All samples collected from the years 1984–2016 were included in the analysis.

A total of 159 patients had pulmonary exacerbation, and viral PCR was performed. Fifty-eight out of 159 patients (36%) had positive viral PCR. Out of the 58 patients, 30 patients were males (52%) and 28 patients (48%) were females. Forty-five out of 58 patients (78%) were alive and 13/58 patients (22%) died. Twenty-five out of 58 patients (43%) were referred from the Eastern region, 11 (19%) from the central region, 10 (17%) from the North, 10 (17%) from the South and only 2 (3%) from the western region.

Methods of sample collection were mostly by nasopharyngeal

aspirate (NPA) in 34 (59%), broncho-alveolar lavage BAL in 5 (9%), and induced sputum from 19 (32%).

Seventy-four positive first viral PCR were obtained from the total 58 PCR samples (some patients had more than one type of viruses). The most frequently reported virus was rhinovirus in 33/74 sample (45%), adenovirus in 12 (16%), human parainfluenza virus (HPIV) in 10 (14%), bocavirus in 6 (8%), enterovirus in 4 (5%), coronavirus OC43 in 4 (5%), human respiratory syncytial virus (RSV) in 2 (3%), Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 1 (1%), influenza A virus in 1 (1%), and influenza B virus in 1 (1%).

Out of 74 viral PCR, 41 (55.4%) were during the colder seasons (October–February) and 33 (44.5%) during the warmer seasons (March–September) (Fig. 1). This shows that the frequency of viral infection was higher during colder seasons.

Rhinovirus infection had two peaks during winter and summer seasons. Out of 33 Rhinovirus cases, 19 (58%) patients were infected in December and 14 (42%) patients in June, respectively (Fig. 1).

During the viral infection, we collected 50 (86%) respiratory samples from the 58 patients for bacterial culture that resulted in 37 positive results. Some patients were infected with more than one type of bacteria. Thirteen out of 37 (35%) were infected with (PA), 8 (22%) with (*S. aureus*), 7 (19%) with (*S. pneumonia*), 3 (8%) with *Escherichia coli* (*E. coli*), 3 (8%) with (*H. influenzae*), 1 (3%) with (*Pseudomonas cepacian*), and 2 (5%) had other bacterial organisms (Table 1).

On follow up over 6 months period, post viral infection, 58 bacterial samples were obtained, resulting in 42 positive culture. 12 out of 42 (29%) were infected with *S. aureus*, 9 (21%) with *Mucoid P. aeruginosa*, 7 (17%) with *H. influenzae*, 2 (5%) with *E. coli*, 5 (12%) with *S. pneumonia*, 1 (2%) with *Klebsiella*, 5 (12%) with other organisms, and 1 (2%) patient was infected with other gram-negative bacteria (Table 1).

3.1. Recurrence of viral infection

Thirteen patients out of 58 (22%) had recurrence of viral infection within 3–6 months. With 21 positive viral PCR, all 13 patients had rhinovirus infection (62%), and Adenovirus was found in 4/21 (19%), Bocavirus in 2 (10%), coronavirus OC43 in 1 (5%), and Enterovirus in 1 (5%).

During the viral recurrence, 13 out of 58 (22%) had bacterial samples taken for culture which resulted in 6 positive bacterial culture. Two of six (33%) were infected with (*Staphylococcus*), 2

(33%) with (*H. influenzae*), 1 (17%) with (*P. aeruginosa*), and 1 (17%) with (*Streptococcus*).

3.2. CFTR mutation

(CFTR) analysis was performed in 57 out of 58 patients. A total of 23 mutations were found. The most common mutations leading to viral infection are 3120+1G>A in Intron 16 in 11/57 patients (19%), 1548delG in Exon 10 in 8 patients (14%), 711+1G>A; Intron 5 in 6 patients (11%) and 2043delG in Exon 13 in 6 patients (11%). The Eastern province had the highest viral infection of 24 out of 57 (42%) (Table 2).

3.3. Pulmonary function test results (PFT)

Twenty patients out of 58 (34%) underwent PFT analysis for the detection and determination of disease severity. Eighteen out of 20 did second PFT analysis and were included in the study. Four out of eighteen (22%) had normal PFT, 1 (6%) obstructive, 2 (11%) restrictive, 11 (61%) combined obstructive and restrictive pattern (Table 3). The severity of the PFT showed that 4/18 (22%) had normal PFT, 4 (22%) mild, 4 (22%) moderate, 2 (11%) severe and 4 (22%) moderately severe (Table 3). Follow up of PFT post viral infection showed no significant difference in the type and the severity of PFT.

Out of the 58 patients, 45 (78%) patients are alive and 13 (22%) died. Three out of 13 patients who died (23%) had *mucoïd P. aeruginosa*, 1 (8%) with *Staphylococcus aureus*, 1 (8%) with *Streptococcus*, 3 (23%) with *E. coli*, and 5 (38%) patients with other organisms.

4. Discussion

The potential significance of respiratory viruses in CF patients was reported only in few studies. Wark et al. showed that viral respiratory tract infections were associated with 65% of the exacerbations of CF cases and it was associated with increased respiratory symptoms [16].

Burns et al. showed that the majority of viruses identified throughout their study were rhinoviruses [17].

Esposito et al. showed that human rhinovirus is the most commonly associated viral infection with CF patients. Rhinovirus detection was significantly associated with the occurrence of CF pulmonary exacerbation [18].

Ramirez IA et al. made a comparison of the pulmonary

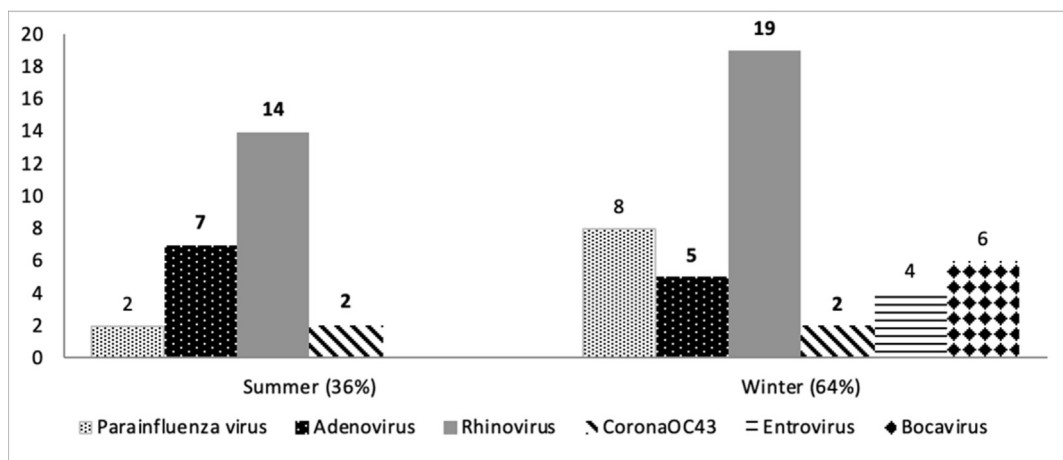


Fig. 1. Comparison of viruses during summer and winter months.

Table 1
Comparison of bacterial colonization during and after viral infection.

Type of Bacteria	First Viral Infection N = 50 (%)	Post Viral Infection N = 50 (%)
All strains of <i>Pseudomonas aeruginosa</i>	13 (35)	9 (21)
<i>Staphylococcus aureus</i>	8 (22)	12 (29)
<i>Streptococcus</i>	7 (19)	5 (12)
<i>Escherichia (E. coli)</i>	3 (8)	2 (5)
<i>Hemophilus influenzae (any species)</i>	3 (8)	7 (17)
<i>Pseudomonas cepacia (Burkholderia cepacia)</i>	1 (3)	-
<i>Klebsiella (any species)</i>	-	1 (2)
Other gram-negative	-	1 (2)
Other bacteria	2 (5)	5 (12)
Grand Total	37	42

The P value is .803.

Table 2
Distribution of mutation for patients with viral infection- 57 patients.

Mutation	East (%)	Central (%)	North (%)	South (%)	West (%)	Total (%)
3120+1G>A; Intron 16	7 (29)	2 (18)	1 (10)	1 (10)	-	11 (19)
1548delG; Exon 10	-	2 (18)	6 (60)	-	-	8 (14)
711+1G>A; Intron 5	-	2 (18)	-	4 (40)	-	6 (11)
2043delG; Exon 13	6 (25)	-	-	-	-	6 (11)
H139L; Exon 4	2 (8)	-	1 (10)	1 (10)	-	4 (7)
I1234V; Exon 19	2 (8)	1 (9)	-	-	-	3 (5)
N1303K; Exon 21	2 (8)	-	-	-	-	2 (4)
L467F; Exon 10	-	1 (9)	-	-	1 (50)	2 (4)
[delta]F508; Exon 10	-	-	1 (9)	1 (10)	-	2 (4)
296 + 12T>C; Intron 2	1(4)	-	-	-	-	1 (2)
G1249E; Exon 20	1 (4)	-	-	-	-	1 (2)
homozygous 712-1 G>C in axon 6.	1 (4)	-	-	-	-	1 (2)
R553X; Exon 11	1 (4)	-	-	-	-	1 (2)
S549R; Exon 11	1 (4)	-	-	-	-	1 (2)
D579G; Exon 12	-	1 (9)	-	-	-	1 (2)
G178R; Exon 5	-	1 (9)	-	-	-	1 (2)
3617G>A exon19	-	-	-	1 (10)	-	1 (2)
R709X; Exon 13	-	-	1 (10)	-	-	1 (2)
c579 + G>A intron5	-	-	-	1 (10)	-	1 (2)
exons: 17a, 17b, 18).	-	-	-	1 (10)	-	1 (2)
M1140K; Exon 18	-	-	-	-	1 (50)	1 (2)
DS459_G461	-	1 (9)	-	-	-	1 (2)
Grand Total	24	11	10	10	2	57

Table 3
Type and severity of PFT.

Type of PFT	During viral infection N = 20 (%)	After viral infection N = 18 (%)
Normal	4 (22.2)	3 (16.6)
Obstructive	1 (5.5)	3 (16.6)
Restrictive	2 (11)	1 (5.5)
Combined	11 (6)	11 (61)
Severity of PFT		
Normal	4 (22)	4 (22)
Mild	4 (22)	4 (22)
Moderate	4 (22)	5(27.7)
Moderately severe	4 (22)	3 (16.6)
Severe	2 (11)	2 (11)

P value for the type of PFT is: 1.000.

P value for the severity of PFT is: 0.848

exacerbations associated with rhinovirus and influenza virus with virus-negative specimens which showed that viruses were associated with immune responses tailored to specific infections [19]. In our study, we showed that rhinovirus was the most prevalent virus. Fortunately, the prevalence of influenza virus in our population was minimum possibly due to the annual routine influenza vaccination.

Armstrong et al. reported that respiratory viruses play a major role in CF hospitalizations and they were associated with acquisition of *P. aeruginosa* although viral infections were self-limited [6].

A respiratory virus infection together with bacterial infection may enhance the inflammation and may lead to chronic bacterial infection with bacterial species such as *P. aeruginosa* [16].

In our study, we reported the respiratory viruses in patients with CF during acute pulmonary exacerbations in 58 out of 159 (36%) patients. We also noted that viral infection increases the prevalence of bacterial infection of specific pathogens such as *H. influenzae* and *S. aureus* (Table 1). We also found that the most frequently reported virus was rhinovirus in 33/74 samples (45%).

Our study showed that viral recurrence occurred within 3–6 months in 13/58 patients (22%) and mainly with Rhinoviral infection. We also noted that *H. influenza* and *S. aureus* were the most commonly reported bacterial pathogen during viral recurrence.

Wang et al. reported that viral infection was associated with bacterial respiratory exacerbation and hospital admissions, in addition to decline in PFT. He also reported an association between the progression of pulmonary disease and the number of annual viral infections. The exact mechanism of this microbial synergism was not known [20].

In our study, a follow-up PFT post viral infection showed no significant difference in the type and the severity of PFT compared to the initial PFT during the viral illness (Table 3). This could be related to the small sample size of those who were able to do the PFT technique.

Emerson et al. showed that patients who were infected with *P. aeruginosa* have 2.6 times increase in the 8-year risk of death [21]. Similarly, in our study, 3/13 patients (23%) who died had infection with mucoid *P. aeruginosa*. This may support the assumption that *P. aeruginosa* may increase the risk of death in patients with CF. In our population, 133 patients died from the whole cystic fibrosis population and 8 patients out of 133 had viral infection (6%).

Further studies should be done to describe the association between specific genetic mutation and viral infection.

5. Conclusion

Viral infections contributed to the increase in morbidity and mortality of CF patients in our population, and rhinovirus was the most common causative agent. Viral infections and viral recurrence increased the prevalence of bacterial infection of specific pathogens such as *H. influenza* and *S. aureus*. Physicians should be aware to prevent progressive lung damage in CF patients by treating the concomitant viral and bacterial infections. Viral infection may be associated with some common CFTR mutations.

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