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Original Article

Is there a role of viral infection in cystic fibrosis exacerbation in children?

Dina H. Hamed[1], Mai Sherif Soliman[2], Ola Soliman Emam[1], Mona Mohsen El Attar[1]

[1]Department of Pediatrics, Children's Hospital, Cairo University, Egypt; [2]Department of Clinical and Chemical Pathology Cairo University, Egypt.

ABSTRACT

Background. Cystic fibrosis (CF) is a degenerative disease distinguished by progressive epithelial secretory gland dysfunction associated with recurrent respiratory tract infections. Despite that bacteria have previously been studied as the main cause of CF airway damage, a strong effect of respiratory viral infections is also now recognized. We aimed to detect the relationship between viral infection and exacerbation in children with cystic fibrosis.

Methods. This is a cross sectional observational study recruiting 60 patients diagnosed as CF following in Cystic Fibrosis Clinic, Children's Hospital, Cairo University, throughout a period of 7 months. Their age ranged from 6 months to 13 years. Patients had nasal swabs and sputum samples obtained when they developed respiratory exacerbations. Multiplex PCR (polymerase chain reaction) technique was used to detect respiratory viruses from nasal swabs.

Results. We detected viruses in 48 patients during exacerbation (80%), the most common virus was rhinovirus in 43.4% of patients, followed by bocavirus in 20%, adenovirus in 13.3%, enterovirus in 10% and human metapneumovirus in 6.7%. Co-infection with double viruses was detected in 10 patients. Bacterial infection was present in 56.7% of patients; the most common organism was *Pseudomonas* in 20% of patients, followed by *Staphylococcus aureus, methicillin resistant Staphylococcus aureus, Klebsiella and Haemophilus influenzae*. CRP was positive in 53.3% of patients. There was a significant relationship between sputum positive bacterial culture and each of influenza A virus, enterovirus and human metapneumovirus.

Conclusions. This study demonstrated that exacerbation in cystic fibrosis may be exaggerated by viral infections such as influenza A and enterovirus necessitating hospitalization which shows the important protective role of vaccination. Also, a strong relationship was detected between some viruses such as enterovirus, human metapneumovirus and influenza and between bacterial infection.

Key words: cystic fibrosis, exacerbation, respiratory viruses, rhinovirus.

Cystic fibrosis (CF) patients frequently describe that colds and other upper respiratory tract infections are the prevalent cause of exacerbations of their respiratory symptoms. Despite this, there is lack of sufficient data in the literature on the role of respiratory viruses in CF lung disease and treatment options for these pathogens.[1]

✉ Dina H. Hamed
dina.alkhtaib@cu.edu.eg

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The role of bacterial pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been greatly investigated for many years whereas the respiratory virus pathogenesis is still not well studied. The availability of new diagnostic molecular tests to detect viral infections has recently expanded the interest in evaluating their effect in some diseases such as CF in children and in adults.[2]

The prevalence of viral respiratory infections varies significantly in the literature, ranging from 5 to 68%.[3]

It is highly detected in children, although a significant number of adult patients with CF also have viral infections, mainly rhinovirus.[4]

Although the specific mechanism by which viral infections may provoke a pulmonary exacerbation in children with CF has not yet been identified, viruses likely play a major role and have to be considered when investigating exacerbations in children.[5]

In this study we aimed to assess the relationship between viral infection and exacerbation in children with cystic fibrosis.

Material and Methods

This was a cross sectional observational study recruiting 60 patients diagnosed as CF, based on clinical manifestations and confirmed by a positive sweat chloride test, coming in acute exacerbation to the Cystic Fibrosis Clinic in Children's Hospital, Cairo University. The study was approved by the Institutional Ethics Committee of Kasr Al Ainy (I-300314) and informed consents were obtained from study subjects and/or their legal guardians before starting.

Inclusion criteria: age range from 6 months to 13 years old, both genders were included.

Full medical history and clinical evaluation with special stress on respiratory manifestations were recorded (fever, exaggerated cough, frequency of exacerbation, need for oxygen, need for hospital admission and intensive care unit admission, type of breathing and oxygen saturation).

Laboratory investigations included C-reactive protein, sputum culture, nasopharyngeal swab.

Blood was collected by venipuncture, allowed to clot and serum was separated by centrifugation at room temperature and was frozen at -20°C. The analysis of all samples was carried out at the laboratory of the Department of Clinical Pathology, Kasr Al Aini Hospital, Cairo University.

The sputum was collected in sterile ice - cream cups after instructing the patient to rinse his/her mouth thoroughly with water and cough forcefully to bring out the mucous from the tracheobronchial tree. Suction was used to collect a sputum sample in patients who were unable to cough. A soft, flexible tube was inserted through the nose and down the throat; and suction was applied for up to 15 seconds to collect the sample. The sputum sample was placed in a container with a growth medium or culture medium. Bacteria that grow on media were detected under a microscope or by chemical tests.

Nasopharyngeal swab samples were in the form of nasopharyngeal swabs taken on viral transport media (VTM).

Nasopharyngeal swab was taken as recommended through inserting the flocked flexible dacron or nylon fiber swab into one of the nostril. The swab is inserted till it reaches half of the distance between the ear lobule and the ala of the nose. The swab is left for few seconds then withdrawn to be put in the VTM containing tube labelled with the patient unique ID. The VTM is prepared inhouse using bovine albumin, HEPES buffer, penicillin and streptomycin in HANK's balanced salt solution. stored in the -70 freezer until processed.

PCR testing for respiratory viruses:

- Viral Nucleic Acid Extraction: Viral nucleic acid extraction was done after centrifugation of the sample for 10-20 min and 200 μl from the sediment taking as starting material using the Biospin Virus RNA Extraction kit (cat number BSC62M1 from Bioflux), according the manufacturer's instructions.
- CDNE was done manually by cDNA Synthesis Premix (SGRT801) from Seegene.
- PCR was done for the following viruses: adenovirus, influenza A virus, influenza B virus, parainfluenza viruses 1–4, rhinovirus, respiratory syncytial virus, bocavirus, human metapneumovirus,

coronavirus 229E, coronavirus NL63, coronavirus OC43, and enterovirus. PCR was done by real-time multiplex PCR using AnyplexTM II RV16 Detection (v1.1) (cat. no. RV7G01Y) supplied by Seegene, operated on a CFX96TM Real-Time PCR Detection System (Bio-Rad).

- Interpretation of the results was done according to the manufacturer's instructions, in addition to automatic analysis using the Seegene viewer software after exporting the run data to it.

Chest X-ray was done for all patients during exacerbation of cystic fibrosis.

Statistical analysis

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data were summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney U tests. For comparing categorical data, chi square (χ^2) test was performed. Exact test was used instead when the expected frequency was less than 5. P-values less than 0.05 were considered statistically significant.

Results

Our study included 38 males (63.3%) and 22 females (36.7%) with a median age of 4 years and range from 6 months to 13 years. Demographic data are shown in Table I.

The most common presentation was exaggerated cough present in all patients, followed by increased wheezing. Sputum culture showed mixed flora in 43.3 % of patients. *Pseudomonas* was the most common organism detected followed by *Klebsiella*. CRP was positive in 53.3% of patients (Table II).

Abnormal chest X-Ray (CXR) was detected in most of the patients in the form of consolidation in 20%, hyperinflation in 23.3%, increased bronchovascular markings in 26.7% and air bronchogram in 10%, while 20% of patients had normal CXR (Table II).

The most common virus detected was rhinovirus in 43.4% of patients, followed by bocavirus in 20%, adenovirus in 13.3%, enterovirus in 10% and human metapneumovirus in 6.7%. Detection of viruses required a semi quantitative method to assess the viral load in the sample (+, ++, +++ as shown in Table III). Double virus was detected in 10 patients (16.7%). None of the included patients had history of influenza vaccination.

A significant relationship was detected between positive bacterial culture and certain viruses as influenza A virus (p value <0.001), enterovirus (p value 0.002) and human metapneumovirus (p value 0.002), but no relationship was noted between double virus and bacteria with a p value of 0.08 (Table IV).

Many viruses were detected in CF patients during exacerbation necessitating hospital admission including: rhinovirus, influenza A, enterovirus, bocavirus and adenovirus. But a significant relationship was only detected between hospital admission and influenza A and enterovirus (Fig 1).

A significant relationship was detected between the presence of influenza A and enterovirus and the need for oxygen (with p values 0.004 and 0.02, respectively).

No significant relationship was detected between the need for intensive care unit (ICU) admission and the 2 viruses detected (rhinovirus and bocavirus) with p values 0.730 and 0.101, respectively.

Statistically significant differences were noted between different age group and influenza A, rhinovirus, enterovirus and adenovirus (with p values 0.036, 0.011, 0.002, 0.018 respectively) (Fig 2), but no relationship was detected in case of bacterial infection (p=0.137).

Table I. Demographic data of the study population (N=60).

Characteristic	n	%
Age groups		
<2 years	18	30.0
2 years to 6 years	24	40.0
>6 years	18	30.0
Gender		
Male	38	63.3
Female	22	36.7
Weight percentile		
Below 5th percentile	44	73.3
From 5th to 95th percentile	16	26.7
Height percentile		
Below 5th percentile	32	53.3
From 5th to 95th percentile	28	46.7
Frequency of exacerbation		
Twice per year	6	10.0
≥3 times per year	12	20.0
≥Once per month	42	70.0
Viral detection: positive	48	80.0
Viral detection: negative	12	20.0
	Mean ± SD	Median (Range)
Age (months)	58.7±41.11	49.5 (6-156)
Weight (kg)	12.73±6.84	11.52 (3-28)
Height (cm)	93.03±23.5	89.5 (55-140)
Age at diagnosis (months)	30.7±39.34	10 (2-132)
Body mass index (kg/m^2)	13.81±1.30	14 (11.2-15.6)
Sweat chloride test (mEq/L)	100.6±24.32	98 (67-155)
Duration of hospitalization (days)	11.14±6.62	8 (3-20)

SD: standard deviation

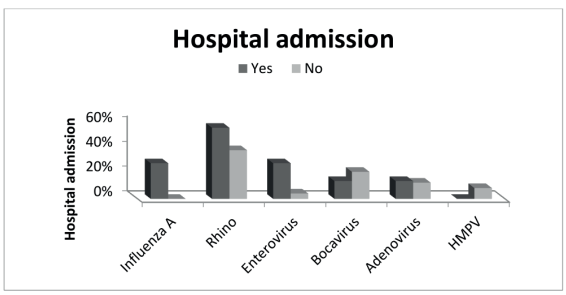

Fig. 1. Relation of viruses with hospital admission.

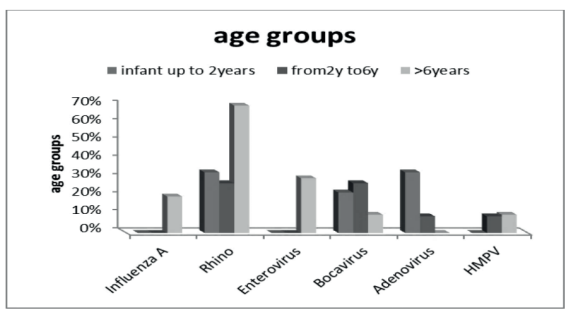

Fig. 2. Relation of age group with virus.

Table II. Clinical, laboratory and radiological parameters of patients during exacerbation (N=60).

Clinical presentation		n	%
Fever		50	83.3
Exaggerated cough		60	100.0
Hospital admission		14	23.3
Need for nasal oxygen		16	26.7
Need for intensive care unit		4	6.7
Increase wheezes		58	96.7
Increased crepitations		22	36.7
Laboratory tests			
Sputum culture	*Staphylococcus aureus*	6	10.0
	Pseudomonas	12	20.0
	MRSA	2	3.3
	Haemophilus influenzae	4	6.7
	Klebsiella	8	13.3
	E. coli	2	3.3
	Mixed flora	26	43.3
CRP	Positive	32	53.3
	Negative	28	46.7
Chest X-ray	Air bronchogram	6	10.0
	Consolidation	12	20.0
	Hyperinflation	14	23.3
	Increased broncho-vascular markings	16	26.7
	Normal	12	20.0

CRP: C-reactive protein, MRSA: methicillin resistant S. aureus

Discussion

In our study, we detected rhinovirus in 43.4% of CF patients during exacerbation. Similarly, it was reported as the most common respiratory virus in exacerbations of CF in many other studies.[2,3,6,7]

In our study, influenza A virus was detected in only 6.7% of patients although no one had a history of influenza vaccination. Similarly, a French study found that its prevalence was 9%.[2]

Also, Asner et al.[8] detected a low proportion of pH1N1 (5.3%) even though it was performed during the larger second wave of the 2009 influenza with a modest pH1N1 vaccine uptake rate of 46.5%.

On the contrary, Wat et al.[9] conducted a study during a typical influenza season and reported higher influenza A and B detection rates (25%) despite a high influenza vaccination uptake of 70%.

We detected adenoviruses in 13.3% of patients. Many studies have reported similar results.[2,8]

Bocavirus has seldom been screened in CF, but in our study, it was detected in 20% of patients. de Almeida et al.[10] and Keravec et al.[11] detected its prevalence to be about 5%.

We detected human metapneumovirus in 6.7% of patients. Another cohort study of older CF children hospitalized for exacerbation, showed that its prevalence reached up to 47.6%.[12]

In addition to rhinoviruses, other enteroviruses can be detected in the airways, leading to respiratory symptoms similar to rhinovirus. In our study, we detected enterovirus in 10% of patients, while in studies by de Almeida et al.[10] and Esposito et al.[13,14] enterovirus prevalence

Table III. Viral load in cystic fibrosis patients.

Virus	Load	n	%
Influenza A	Undetected	56	93.3
	Detected +	2	3.3
	Detected ++	2	3.3
	Detected+++	0	0.0
Influenza B	Undetected	60	100.0
Rhinovirus	Undetected	34	56.7
	Detected +	4	6.7
	Detected ++	22	36.7
	Detected+++	0	0.0
Respiratory syncytial virus-A	Undetected	60	100.0
Respiratory syncytial virus -B	Undetected	60	100.0
Enterovirus	Undetected	54	90.0
	Detected +	2	3.3
	Detected ++	4	6.7
	Detected+++	0	0.0
Bocavirus	Undetected	48	80.0
	Detected +	8	13.3
	Detected ++	4	6.7
	Detected+++	0	0.0
Adenovirus	Undetected	52	86.7
	Detected +	4	6.7
	Detected ++	2	3.3
	Detected+++	2	3.3
Human metapneumovirus	Undetected	56	93.3
	Detected +	0	0.0
	Detected ++	4	6.7
	Detected+++	0	0.0

mostly ranged between 3.2 and 7.75%, but can reach 29.4% or 35%, as reported in two 5-month studies.[8,15]

Viral coinfection is quite common in the CF respiratory tract, it involves at least two viruses, and sometimes more. We detected viral coinfection in 16.7% of patients. All of them were double viruses (rhinovirus with enterovirus, rhinovirus with bocavirus, rhinovirus with adenovirus and adenovirus with bocavirus). Other studies showed that viral coinfection rates ranged from 0 to 34.6%.[4,8] In Miró-Ca˜nísa et al.[16] study, the most common virus coinfection was rhinovirus plus adenovirus (6/20) and rhinovirus plus enterovirus (4/20).

A statistically significant relationship was noted between different age groups and some viruses, but no relation was detected in case of bacterial infection. Similarly, Asner et al.[8] detected that virus positive patients during CF pulmonary exacerbation were significantly younger than virus negative patients.

We detected a relationship between bacteria and each of influenza virus, enterovirus and human metapneumovirus which highlight the possibility that respiratory virus infections could increase the severity of infection.

Wat et al.[9] didn't find statistical differences between the viral and non-viral groups for bacteria isolation (p=0.909). Charles and

Table IV. Relationship between viral and bacterial culture.

	Sputum culture														P value
	S. aureus (n=6)		*Pseudomonas* (n=12)		*MRSA* (n=2)		Mixed flora (n=26)		*Klebsiella* (n=8)		*H. influenzae* (n=4)		*E. coli* (n=2)		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Influenza A (n=4)	2	33.3	-	-	-	-	-	-	-	-	-	-	2	100.0	<0.001
Rhinovirus (n=26)	2	33.3	4	33.3	-	-	12	46.2	4	50.0	2	50.0	2	100.0	0.169
Enterovirus (n=6)	-	-	4	33.3	-	-	-	-	-	-	-	-	2	100.0	0.002
Bocavirus (n=12)	-	-	-	-	-	-	10	38.5	2	25.0	-	-	-	-	0.427
Adenovirus (n=8)	-	-	4	33.3	-	-	4	15.4	-	-	-	-	-	-	0.721
Human metapneumovirus (n=4)	-	-	2	16.7	2	100.0	-	-	-	-	-	-	-	-	0.002

MRSA: methicillin resistant S. aureus

Esther's study[17], did not detect a relationship between respiratory virus pathogen status and the frequency of typical CF pathogens such as *Staphylococcus aureus* or *Pseudomonas aeruginosa*.

Many viruses were detected in CF patients during exacerbation necessitating hospital admission. Armstrong et al.[18] demonstrated that respiratory viruses play a key role in CF hospitalizations and they were associated with acquisition of *Pseudomonas aeruginosa* although viral infections were self-limited. Wang et al.[19] have shown the association between viral infection and bacterial respiratory exacerbation, and hospital admissions. Another study detected respiratory viruses in 36% of CF patients during acute pulmonary exacerbations and showed that viral infection increases the prevalence of bacterial infection of specific pathogens such as *Haemophilus influenzae* and *Staphylococcus aureus*.[6] Also, Garcia et al.[12] has shown that *human metapneumovirus* behaves similarly to respiratory syncytial virus in CF and leads to an increased risk of hospitalization and exacerbation.

No significant relationship was detected between the need for ICU admission and the 2 viruses detected (rhinovirus and bocavirus). A study detected that the only discriminatory feature of the children who were rhinovirus positive was lower oxygen saturation.[3]

In conclusion, exacerbation in cystic fibrosis may be exaggerated by viral infections such as influenza A and enterovirus necessitating hospitalization which demonstrate the important role of vaccination and the role of awareness of treating the concomitant viral and bacterial infection. Also, a strong relationship was detected between some viruses such as enterovirus, human metapneumovirus and influenza and between bacterial infection.

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Ethical approval

The study was approved by the Institutional Ethics Committee of Kasr Al Ainy on March 2016 (I-300314) and informed consents were obtained from study subjects and/or their legal guardians before starting.

Author contribution

The authors confirm contribution to the paper as follows: study design: DHH, MSS, MMEA; data collection: OSE; analysis and interpretation of the results: MSS, draft manuscript preparation: DHH, OSE, MMEA. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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