Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original research doi: 10.12980/JCLM.3.2015J5-49

Distribution of respiratory viruses which cause lower respiratory tract infection in pediatric age group

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 25 May 2015
Accepted 1 Jun 2015
Available online 11 Jun 2015

Keywords:
Respiratory
Virus
Pediatrics
Lower respiratory tract

ABSTRACT

Objective: To determine the appropriate treatment regimen and the clinical course of the lower respiratory tract infections(RTIs) and to detect the common viral causes of lower RTIs.

Methods: The present study included a total of 255 pediatric patients aged less than 7 years old and admitted to the Department of Pediatrics of Rize Training and Research Hospital between January 2014 and January 2015 with clinical pre-diagnosis of lower RTI. Nasopharyngeal swab specimens collected from these patients were tested for viral pathogens by using multiplex RT-PCR kit the ResPlex II plus Panel PRE (Qiagen, Germany).

Results: A total of 212 out of 255 (83.1%) specimens revealed positive for one or more viral pathogens. The most common detected pathogens were respiratory syncytial virus (RSV) A/B in 110 samples (43.1%), rhinovirus in 51 samples (20.0%), adenovirus in 36 samples (14.1%), influenzae virus A in 32 samples (12.5%), and coronavirus in 24 samples (9.4%). In 76 samples (29.8%), more than one viral pathogen were detected. RSV was seen in more than 50% patients in the first 2 years. RSV was the most common pathogen in each year of the first 5 years but rhinovirus, influenza A and adenovirus were seen more than RSV after the fifth year. A total of 95.8% of the viral detections were seen between November and April without a significant peak amongst these months. The distribution of the pathogens by months of the year showed no significance.

Conclusions: These findings can contribute to epidemiological data of Turkey. Detection of the viral pathogens causing lower RTIs can be critical in management of the disease, decrease inappropriate antibiotic treatment, and lower the morbidity and mortality rates in such diseases.

1. Introduction

Lower respiratory tract infections (RTIs) are common health problems particularly in children. More than 80% of lower RTIs are caused by viruses such as rhinoviruses, respiratory syncytial viruses (RSVs), adenoviruses, influenzae A and B, parainfluenzae viruses (PIVs) type I, II, III, and IV, coronaviruses (CoVs), and human metapneumoviruses (HMPVs)[1-3].

RSVs, known as the most common cause of bronchiolitis and pneumoniae in infants throughout the world, are single-stranded enveloped RNA viruses, as well as members of the family Paramyxoviridae[4]. RSVs are divided into two subgroups as RSV A and B depending on the antigenic and genetic variety[4]. Rhinoviruses are members of enteroviruses, and one of the most common causes of upper RTIs. Adenoviruses are double-stranded non-enveloped DNA viruses, and cause gastroenteritis, cystitis, meningoencephalitis as well as RTIs. Influenzae viruses are members of Orthomyxoviridae, and are single-stranded and eight-segmented RNA viruses. They are well-known viruses with numerous antigenic variety caused by antigenic shifts amongst the segments. HMPVs are also members of Paramyxoviridae and amongst the causative agents of RTIs. CoVs are single-stranded RNA viruses and cause cardiovascular, neurological and RTIs[1,2,4].

Detection of viral causes of RTIs had not been widely used due to lack of requirement of antiviral treatment, or lack of numerous antiviral agents. However, in recent years, development and common usage of new antiviral agents have couraged the determination of viruses in cases of RTIs. In addition, epidemiological researches increased the requirement of viral detection[2,3,5].

RT-PCR is the most common used method in detection of viral

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causes of RTIs because this method is reported to be a rapid, highly sensitive and specific way in the differential diagnosis of these cases. In addition, RT-PCR is easy to perform and require no experienced staff[3,5].

In the present study, it was aimed to determine the distribution of viral causes in children admitted to the Department of Pediatrics of Rize Training and Research Hospital between January 2014 and January 2015 with pre-diagnosis of RTI.

2. Materials and methods

2.1. Patients

The present study included a total of 255 pediatric patients aged less than 7 years old and admitted to the Department of Pediatrics of Rize Training and Research Hospital between January 2014 and January 2015 with clinical pre-diagnosis of lower RTI. Nasopharyngeal swab specimens collected from these patients were transferred to the microbiology laboratory and stored at -20 ℃ until tested.

2.2. RT-PCR

The nasopharyngeal samples were tested for respiratory viral pathogen by using the ResPlex II plus Panel PRE (Qiagen, Germany) that covered viruses such as rhinoviruses, H1N1, RSV A and B, influenzae A and B, adenoviruses, CoVs (NL63, HKU1, 229E, and OC43), PIV1, PIV2, PIV3, PIV4, HMPV A and B, human parechoviruses, enteroviruses, human bocaviruses, and a bacteria, *Mycoplasma pneumoniae* (*M. pneumoniae*).

The method was performed following the manufacturer's instructions. First of all, nucleic acids were extracted from the specimen by using the Q-card EZ1 virus mini kit (Qiagen, Germany) in the EZ1 extraction system. The amplification was done by using the ABI-7500 RT-PCR system[2]. The amplification product was then detected and identified by using a suspension bead array for multiplex hybridization in the LiquiChip 200 Workstation with the QIAplex MDD software (Qiagen, Germany). A positive result was defined by using a cut-off median fluorescence intensity of 250 or the mean plus 2 standard deviations of the negative controls[6].

2.3. Statistical analysis

Descriptive variables were expressed as numbers and percentages. *Chi*-square or Fisher's exact tests were used for comparison between the groups in terms of categorical variables, wherever appropriate. A P value less than 0.05 was considered statistically significant.

3. Results

A total of 212 out of 255 (83.1%) specimens revealed positive for one or more viral pathogens. Amongst the positive samples, the male/female ratio was 132/80, and the difference was not statistically significant (P = 0.175). However, more than one pathogen was detected in 53 male and in 23 female patients with statistical significance (P = 0.047) (Table 1). The mean age was (1.93 ± 1.88) years (minimum: 2 months, maximum: 7 years).

Table 1
Distribution of the pathogens by gender [*n* (%)].

Gender	Virus positive	> 1 pathogens detected	Total number
Girl	80 (79.2)	23 (22.8)	101
Boy	132 (85.7)	53 (34.4)	154
P	0.175	0.047	

The most common detected pathogens were RSV A/B in 110 samples (43.1%), rhinovirus in 51 samples (20.0%), adenovirus in 36 samples (14.1%), influenzae virus A in 32 samples (12.5%), and CoV in 24 samples (9.4%). *M. pneumoniae* was detected in two samples, and influenzae virus B was found in one sample. In addition, no influenzae virus H1N1 or human parechovirus were detected in the samples (Table 2). In 76 samples (29.8%), more than one viral pathogens were detected (Table 3).

Table 2
Frequencies of the viral pathogens in samples with one or more pathogens.

Pathogens	Any count of pathogens [*n* (%)]	Only 1 pathogen (%)	> 1 pathogens (%)
RSV A/B	110 (43.1)	60 (33.5)	51 (65.8)
Rhinovirus	51 (20.0)	18 (9.5)	34 (44.7)
Adenovirus	36 (14.1)	11 (6.1)	26 (32.9)
Influenzae virus A	32 (12.5)	21 (11.7)	12 (14.5)
CoV	24 (9.4)	4 (2.2)	20 (26.3)
Bocavirus	15 (5.9)	9 (4.5)	7 (9.2)
HMPV A	15 (5.9)	9 (5.0)	7 (7.9)
Enterovirus	11 (4.3)	2 (0.6)	11 (13.2)
PIV	9 (3.5)	6 (2.8)	5 (5.3)
Others*	3 (1.2)	0 (0.0)	1 (1.2)

No influenzae virus H1N1 or human parechovirus were detected in the samples; *Others: Influenzae virus B (in one sample) and *M. pneumoniae* (in 2 samples).

Table 3
Distribution of the viral detection test results by age.

Age (year)	Total test samples	Any viral pathogen [*n* (%)]		> 1 pathogens [*n* (%)]	
< 1	43	34	79.1	15	34.9
1	105	93	88.6	31	29.6
2	45	38	84.5	9	20.0
3	20	15	75.0	7	35.0
4	9	7	77.8	2	22.3
5	11	9	81.9	5	45.5
6	9	8	88.9	4	44.5
7	13	8	61.6	3	23.1
Total	255	212	83.2	76	29.8

RSV was seen in more than 50% patients ≤ 1 year. Influenzae virus was seen in all ages in rates between 10%-45% but no case was positive in patients < 1 years old amongst 43 samples. Rhinoviruses and adenovirus were seen in all ages without a significant peak in rates between 5%-55%. RSV was the most common pathogen in patients ≤ 4 years but rhinoviruses, influenzae A, and adenovirus were seen more than RSV in patients after the fifth year (Table 4).

There were statistically significant relationship between detection of more than one pathogen and detection of rhinoviruses (P < 0.001), adenovirus (P < 0.001), enterovirus (P < 0.001), CoV (P < 0.001) and RSV A/B (P = 0.002). Amongst these samples, the most frequent pathogens were RSV A/B (65.8%), rhinoviruses (44.7%), adenovirus (32.9%) and CoV (26.3%). Amongst the samples with one pathogen, the most common viruses were RSV A/B (44.1%), influenzae A (15.4%), and rhinoviruses (12.5%) (Table 5).

Table 4
Distribution of the pathogens by age (%).

Age (year)	Number of total samples	Influenzae A	HMPV A/B	Rhinovirus	RSV A/B	Bocavirus	Adenovirus	Enterovirus	PIV	CoV
< 1	43	0.0	0.0	21.0	51.2	0.0	11.7	14.0	7.0	11.7
1	105	12.4	7.7	19.1	52.4	3.9	13.4	1.0	2.0	10.5
2	45	11.2	6.7	17.8	40.0	8.9	13.4	4.5	4.5	8.9
3	20	20.0	5.0	20.0	35.0	25.0	5.0	5.0	5.0	5.0
4	9	11.2	0.0	22.3	33.4	0.0	11.2	0.0	11.2	0.0
5	11	18.2	0.0	36.4	9.1	9.1	54.6	9.1	0.0	9.1
6	9	44.5	11.2	33.4	22.3	11.2	11.2	0.0	0.0	11.2
7	13	23.1	15.4	7.7	15.4	0.0	15.4	0.0	0.0	7.7
Total	255	12.5	5.9	20.0	43.1	5.9	14.1	4.3	3.5	9.4

Table 5
Relationship between the viral pathogens and detection of one or more pathogens (%).

Pathogens	Number of positive samples	Influenzae A	HMPV A/B	Rhinovirus	RSV A/B	Bocavirus	Adenovirus	Enterovirus	PIV	CoV
1 pathogen	136	15.4	9	12.5	44.1	5.9	8.1	0.7	3.7	2.9
> 1 pathogen	76	14.5	6	44.7	65.8	9.2	32.9	13.2	5.3	26.3
P		0.85	0.728	< 0.001	0.002	0.365	< 0.001	< 0.001	0.583	< 0.001

In samples which more than one pathogen was detected, influenzae virus A and RSV A/B were seen in just three samples with statistically significance ($P < 0.001$). Similarly, RSV A/B and bocavirus were seen in just two samples ($P < 0.016$). In contrast, rhinoviruses and enteroviruses were found together in significantly higher number of samples than presence alone ($P < 0.001$) (Table 5).

A total of 92.0% of the viral detections were seen between October and March without a significant peak amongst these months (Table 6). The distribution of the pathogens by month showed no significance. However, RSV showed peaks in December and March.

Table 6
Distribution of number of positive samples by month.

Months	Number of total samples	Number of positive samples [*n* (%)]
January - March	182	156 (85.7)
April - June	21	15 (71.4)
July - September	5	2 (40.0)
October - December	47	39 (83.0)

4. Discussion

Lower RTIs can cause high morbidity and mortality rates particularly in developing countries[7]. Bacterial pathogens cause more severe clinical manifestations than viral ones. Detection of the causative agent can play an important role in determining the appropriate treatment regimen and the clinical course of the patient[7].

Development of commercial RT-PCR-based systems containing multiple target primers made it easy to detect the common viral causes of lower RTIs. Though the low sensitivities and specificities are reported between about 10%-75%, these multiplex RT-PCR kits have been widely used in diagnosis[2,3,5]. The low sensitivity rates of the kits have been frequently reported for H1N1-p, influenzae A, enterovirus, CoV (OC43), and RSV depending on the reference standards due to lack of careful optimization, low viral load, and the patient population of the study. For instance, the kit sensitivity was found to be high when cell culture was accepted as the main standard but was reported to be low in the case that monoplex PCR was used as the standard[2,8,9]. In the present study, the sensitivity or specificity rates were not calculated due to lack of usage of any main standard methods. In addition, viral loads were not determined in the study.

RSV is known to be the most common causative viral agent in lower RTIs. It causes bronchiolitis and pneumoniae. RSV infections are mostly seen in infants less than 1 year old and are reported to cause severe clinical manifestations[7]. Yarkin *et al.* found the most common agent as RSV with a rate of 24.7%[7]. Akcali *et al.* also found the most common agents as RSV (61%) and rhinoviruses (35%)[10]. Some authors reported the most frequent virus as RSV (29.5%, 31.5%, 44.7%, 56.3%, and 35%, respectively)[11-15]. In contrast, Sancakli *et al.* and Ecemis *et al.* found the most common pathogen as rhinoviruses (26.4% and 25.4%, respectively)[16,17]. Ünüvar *et al.* reported the most common viral agent as influenzae among all patients[18]. However, they reported that RSV and influenzae were both the most common viruses in children less than 5 years old. They added that RSV frequency decreased significantly in above 5-year-old children. In the present study, RSV was the most frequent viral pathogen in the patients less than 5 years old. However, rhinoviruses, influenzae A and adenovirus were found to be more frequent than RSV in patients over the age of five. In addition, RSV was seen in more than 50% patients less than 2 years old. No influenzae virus A was detected in children in the first year though this agent was found in all ages in various rates. In addition, rhinoviruses and adenovirus were detected in all ages without a significant peak. These findings support that viral agent frequency distribution changes with age even in childhood ages.

Mixed viral and bacterial infections have commonly been seen in rates between 25%-50%, and affect the prognosis worse. Mixed viral infection rate was reported to be seen between 3%-8%[7]. In the present study, mixed infection rate was 29.9% amongst all samples, and was 35.8% (76/212) amongst the samples in which any pathogens were detected. Amongst these samples, the most frequent pathogens were RSV A/B (65.8%), rhinoviruses (44.7%), adenovirus (32.9%), and CoV (26.3%). Amongst the samples with one pathogen, the most common viruses were RSV A/B (33.5%), influenzae A (11.7%), and rhinoviruses (9.5%). These findings suggest that some viruses trend to infect or colonize the patient in case of presence of some other viruses. Significant increase in frequencies of CoV and enterovirus in multi-pathogen samples can support this trend for these viruses. In contrast,

frequencies of some viruses such as bocavirus, parainfluenzae virus and HMPV A did not change significantly by number of pathogen types present in the sample.

In addition, RSV A/B was found together with influenzae virus A and bocavirus in significantly low number of samples. This can be explained as i) virus interference between these agents, ii) similar targets in the host, and iii) a technical problem in the multiplex PCR causing false negative result. In contrast, rhinoviruses and enteroviruses were found together in significantly higher number of samples. This finding can be stated as the presence of one of these two viruses make it easy for the other one to infect the patient.

It is reported that most of the lower RTI cases are seen in the first and the second year of children, respectively. Yarkin *et al.* reported that 76.4% of patients with lower RTI were less than 1 year old[7]. Similarly was shown in the present study.

Nasopharyngeal swab samples were used in the present study as the manufacturer of the kit recommended. In addition, nasopharyngeal specimens were reported to reveal reliable results as nasal aspirates in detecting viral agents[18]. Yarkin *et al.* reported that RSV infections made a peak in April, the "rainy" month, but the other viruses were seen in each month of the year[7]. Bayrakdar *et al.* found that RSVs, adenoviruses, HMPVs, enteroviruses and most of parainfluenzae viruses and CoVs were seen between fall and spring[4]. Gülen *et al.* reported that most of influenzae was seen in November and December, and RSV between December and February[19]. In the present study, more than 90% of the viral pathogens were detected between October and March. The distribution of the pathogens by month showed no significance. However, RSV showed peaks in December and March. These findings support the frequency increase of the viral pathogens in the "cold" or "rainy" months.

In conclusion, we found that RSV, rhinoviruses, adenovirus, and influenzae virus A were the most common viral pathogens in children with lower RTI, respectively in terms of frequency. RSV was the most common in children below 5 years old, and the frequencies of the common pathogens were similar to each other over the age of five. These findings can contribute to epidemiological data of Turkey. Detection of the viral pathogens causing lower RTIs can be critical in management of the disease, decrease inappropriate antibiotic treatment, and lower the morbidity and mortality rates in such diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors would be thankful to Training and Research Hospital Microbiology Laboratory and Department of Children Health and Diseases, Faculty of Medicine, University of Recep Tayyip Erdogan, for supplying data.

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