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The frequency of respiratory tract viral agents in children with lower respiratory tract infections

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Summary

Aim: Lower respiratory tract infections (LRTI) have high morbidity rates in children. In this study, it was aimed to investigate the prevalence of respiratory viruses in children with LRTI symptoms.

Material and Method: A total of 160 children who were diagnosed with LRTI between October 2009 and March 2010 were included in the study. The presence of respiratory syncytial virus (RSV) (A+B), influenza virus (A+B), parainfluenza virus (PIV) (1, 2, 3, 4), human metapneumovirus, rhinovirus and coronavirus (OC43+229E) in throat swab samples were investigated by real-time PCR The RealAccurateTM Respiratory RT PCR Kit (PathoFinder B.V., Netherlands).

Results: In 67 samples (41.8%), at least one virus which could cause acute respiratory tract infection was found. Overall, RSV was the most frequently identified virus (52.2%), followed by rhinovirus (26.8%), coronavirus (5%), metapneumovirus (2.9%) and PIV 1 (1.4%). As the other viral agents, coronavirus was detected in 4 samples (5%), hMPV was detected in 2 samples (2.9%) and PIV was detected in 1 sample (1.4%). When the frequency of coinfections was evaluated, RSV- Rhinovirus association was found in 4 samples, RSV-Coronavirus association was found in 1 sample, Rhinovirus-Coronavirus association was found in 1 sample and RSV-Rhinovirus-Coronavirus association was found in 1 sample.

Conclusions: In 41.8% of the study group, a viral factor responsible for the clinical signs was detected. For that reason, rapid and sensitive diagnosis of viruses which lead to respiratory infections will guide the clinician for avoidance of redundant antibiotic therapy and preventing viral hospital infections. *(Turk Arch Ped 2013; 48: 215-220)*

Key words: Children, respiratory tract infections, real-time PCR, viruses

Introduction

Lower respiratory tract infections (LRTI) are an important cause of morbidity and mortality in the childhood. In 80-90% of lower respiratory tract infections, the main agents include rhinoviruses, respiratory syncytial virus (RSV), influenza A and B, parainfluenza viruses (PIV type 1,2,3) and adenoviruses (1). In recent years, new agents have been added to this list with the following order; firstly enterovirus, PIV type 4, mimivirus, afterwards "human metapneumovirüs" (hMPV) in 2001, coronaviruses (CoV, HCoV NL63 and HKU1 which are responsible of SARS) in 2003, human Bocavirus (HBoV), parvovirus type 4 and 5 in 2005 and human Polyomavirus KI (KIV) and WU (WUV) in 2007 (2).

The diversity of the viruses leading to respiratory tract infection and difficulty in detecting viruses rendered regular investigation of viral etiologic diagnosis unnecessary for a long time and these investigations were performed for epidemiological aim previously. However, introduction of gradually increasing number of antiviral drugs in treatment of respiratory tract infections, understanding that these viruses are among the agents of important nasocomial infections, consciousness about the fact that early viral diagnosis will prevent unnecessary antibiotic useage generated the necessity of timely diagnosis of the agents of respiratory tract infections as in other viral diseases (3).

Reverse transcriptase-Polymerase Chain Reaction (RT-PCR) has ranked first among the molecular methods

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as a rapid, sensitive and specific method in detecting of respiratory tract viruses (4). In recent years, a new method called real-time PCR has been developed by combining the devices used to provide heat cycles in PCR reactions (thermocyclers) with sensitive measurement devices and has found a wide area of usage.

In our country, there are very few studies investigating many viruses in relation with the frequency of respiratory tract viruses in LRTIs in the childhood. Therefore, it was aimed to determine the frequency of respiratory viral agents in children with LRTI symptoms in our region in this study using real time PCR which is a rapid and sensitive method.

Material and Method

Patient group and clinical samples

160 children (60 girls, 100 boys) aged between 0 and 10 years (mean: 14,6 months) who presented to Tepecik Education and Research Hospital Pediatric Emergency Department and Pediatrics Outpatient Clinic between October 2009 and March 2010 with LRTI symptoms were included in the study. The diagnosis of LRTI was made with clinical findings including fine rales, rhonchi, respiratory distress and fever and infiltration findings on lung graphy radiologically. Throat dacron swab samples obtained from these patients were transferred to the laboratory in viral transportation media (Universal transport medium (UTM) kit, Copan Diagnostics, Brescia, Italy) in a few hours in accordance with cold chain rules and were kept at -80°C until laboratory tests were performed. No culture was performed for bacteriologic examination, since the study aimed to determine the frequency of viral agents. RT-PCR test was applied to the samples in Celal Bayar University Medical Faculty, Department of Medical Biology, Molecular Biology Laboratory. Our prospective study was confirmed by Celal Bayar University Medical Ethics Committee (Number: 2009-0031) and informed consent was obtained from the legal representatives of the patients. The demographic data, clinical findings, examination findings, radiological findings and laboratory data of the patients included in the study were recorded in the forms prepared.

Real-time reverse transcriptase polimerase chain reaction test (rRT-PCR)

After RNA extraction was performed from the clinical samples, a commertial rRT-PCR method [The RealAccurateTM Respiratory RT PCR Kit (PathoFinder B.V., Holland)] was used to determine RNAs of the respiratory viruses and fluorescent radiation was measured by ABI 7500 system (Applied Biosystems, Foster City, USA) to determine amplicons. Target RNAs specific for viruses found in the kit were used as positive control and "RNase free" water was used as negative control.

The RealAccurate RT PCR Kit (PathoFinder, B.V., Holland) detects 12 RNA viruses [RSV (A+B), influenza virus (A+B), parainfluenza virus (PIV) (1, 2, 3, 4), hMPV, rhinovirus ve coronavirus (OC43+229E)] which are responsible of approximately 90% of respiratory diseases by real time PCR. The kit is a ready-to-use set containing the primers which can detect each of these viruses and TaqMan probes. Since viral nucleic acid is RNA, real time PCR includes a reverse transcription stage to form cDNA. Afterwards, cDNA is reproduced by real-time PCR using virus-specific primer/probe compatibility. Amplicons are detected with measurement of flourescent radiation during polymerase chain reaction.

Results

The rates of positivity of respiratory viruses found in clinical samples

In 67 of the samples (41.8%), at least one viral agent which could lead to LRTI findings was found. Respiratory syncytial virus was the most common virus with a rate of 61.2%. This was followed by rhinoviruses with a rate of 35.8%. The other viral agents found included coronavirus in four samples, hMPV in two samples and PIV in one sample. The distribution of the viruses found by ages is shown in Figure 1.

RSV-rhinovirus association was found in four samples, RSV-coronavirus association was found in one sample, rhinovirus-coronavirus association was found in one sample and RSV-rhinovirus-coronavirus association was found in one sample.

The distribution of the patients with LTRI in whom at least one viral agent was found by months

Seven of the patients who were included in the study between October 2009 and March 2010 presented to the outpatient clinic in December, 37 presented in January, 54 presented in February and 62 presented in March.

When the months of positivity by agents were examined, RSV was found most commonly in March and January and rhinovirus was found most commonly in January and March. Coronavirus was found in patients who presented in February and hMPV was found in patients who presented in February and March.

Totally, positivity in the samples was observed at the highest levels in March (34,6%).

Clinical and physical examination findings in patients in whom at least one viral agent was found

Cough was the most common finding in 64 patients with a rate of 95,5% among the physical examination findings in patients in whom at least one viral agent was found and the patients had cough which had lasted for a mean time of 5.8 days. This was followed by nasal congestion in 42 (62.7%) patients and fever in 36 patients (53.7%). The patients had fever which had lasted for a mean time of 4.1 days. Wheezing was found in 29 patients (43.3%) (Figure 2).

Pathologic lung sounds were found in 66 (98.5%) of 67 positive patients. Lung graphy was evaluated in 58 of the

Table 1. Studies in which lower respiratory viral agents were investigated in symptomatic children in our country and the distribution of the agents detected

	Rhinovirüs (%)	RSV (%)	hMPV (%)	Influenza A (%)	Adenovirus (%)	Bocavirüs (%)	Coronavirus (%)	PIV (%)	Enterovirus (%)
Sancaklı et al.	26.4	10.3	6.9	(%)	2.3	2.3	2.3	3.4	1.1
Yüksel et al..		31.5		23.6	31.5			26.3	
Tanır et al.		44.7							
Yılmaz et al.		35							
Yılmaz et al.		39							
Kanra et al.		29.5							

RSV : . respiratory syncytial virus. Hmpv: human metapneumovirus. PIV: parainfluenza virus

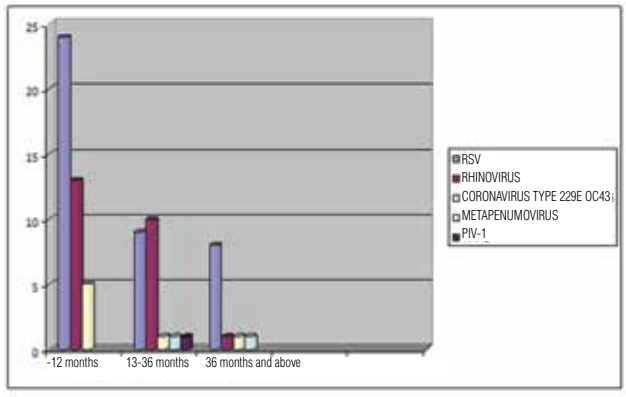

Figure 1. Distribution of the viruses by age in positive samples

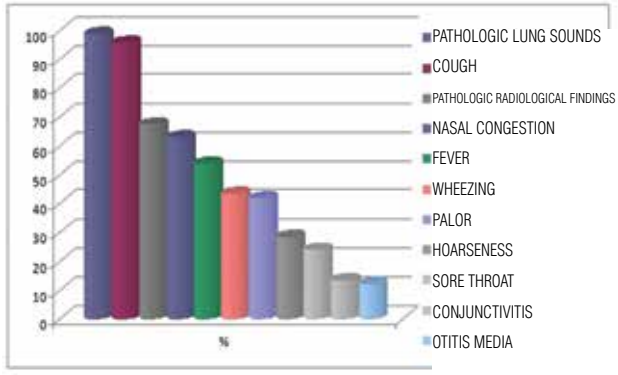

Figure 2. Clinical and physical examination findings in patients who were found to have at least one

patients and radiological findings related with lung disease were found in 39 (67.2%) of them. The mean respiratory rate of the patients was 43.8/min. The mean PO2 was found to be 99.3 in 52 patients in whom PO2 value was measured.

Discussion

Lower respiratory tract infections are blamed for the mortality in at least 4 million children below the age of five in developed and developing countries. This number constitutes approximately 30% of the mortality rate in the childhood (5,6). Bacteriae and mixed infections play a greater role in the mortality rate related with lower respiratory tract infections compared to viral agents. While the mortality rate related with viral pneumonia is 1-7.3% in developing countries, it is 10-14% for bacterial pneumonia and 16-18% for mixed infections (7).

Viruses are involved in 80-90% of respiratory tract infections (1). Because of the diversity of viruses which cause to infections in the respiratory tract and the fact that medical treatment for these viruses is far from general acceptance, use of diagnostic methods directed to

reproduction of nucleic acids in respiratory tract infections caused by various virus families including paramyxoviruses, orthomyxoviruses, adenoviruses, coronaviruses or picornaviruses remained behind compared to other viral infections (8).

Currently, the most common "conventional" methods in detecting these respiratory tract pathogens include obtaining the virus in cell culture (viral culture) and/or detection of viral antigens by the immunoflourescence (IF) method. Viral culture is considered "gold standard" in detection of these pathogens, but this method is usually time consuming and the results can not be obtained before 14 days. Detection of viral antigen by immunoflourescence gives a rapid result, but the sensitivity of this method in detecting some viruses is frequently limited and confirmation of the results by further tests including viral culture is required. Although combined use of both techniques causes to an increase in the rate of positive results, a certain number of samples may be negative even if both methods are used despite clinical and epidemiological suspicion of viral infection.

All these limitations intensified the interest on development of new nucleic acid-based methods. "Real-time reverse transcriptase polimerase chain reaction" test is a rapid, sensitive and specific method in detecting respiratory tract viruses and has found a wide area of usage (4). In addition to determining 30-40% more viral infections compared to IF and culture, this method has provided the diagnosis of newer pathogens including bocavirus and coronavirus which can not be detected by IF and culture in addition to classical respiratory viruses (9). The sensitivity and specificity of RT-PCR have been reported to be 94.4% and 100% for RSV, 100% and 91.3% for rhinovirus, 98% and 98% for influenza virus, 100% and 95% for PIV and 96% and 98.8% for hMPV, respectively (10).

In recent years, a new method called real-time PCR has been developed by combining the devices used to provide heat cycles in PCR reactions (thermocyclers) with sensitive measurement devices. This method eliminates post-PCR procedures. This provides more sensitive results, decreases the risk of contamination and excludes post-PCR procedures which are potential sources of error. On the other hand, real-time PCR determines a more wider dynamic range of 108-101 compared to "conventional" PCR.

Our study is the first study in our region investigating the respiratory viruses which have a significant morbidity and mortality rate in the chilhood age group using molecular methods in symptomatic children. 67 (41.8%) of 160 samples examined were found to be positive at least for one agent. Respiratory scyncytial virus was the most commonly found virus with a rate of 61.2% and this was followed by rhinoviruses with a rate of 35,8%. The other viral agents found included coronavirus in four samples, hMPV in two samples and PIV in one sample. When we examined the studies performed on this issue in our country, we noted that the studies which used molecular methods in the childhood age group and examined such a wide range of viral state were only thesis studies and there was no published study related with this issue in the literature. In the study performed by Sancaklı et al. (11) in 2012 in which 87 subjects with bronchiolitis, bronchopneumonia and pneumonia were included, a virus was determined by PCR in 59 of the subjects (67.8%). Rhinovirus was found in 26.4% of these, RSV A-B was found in 10.3%, hMPV was found in 6,9%, influenza A was found in 3.4%, adenovirus was found in 2.3%, bocavirus was found in 2.3%, coronavirus was found in 2.3%, PIV1-3 was found in 3.4%, enterovirus was found in 1,1%, rhinovirus +RSVA-B was found in 4.6%, adenovirus+RSV was found in 2.3%, coronavirus+bocavirus was found in 1.1% and rhinovirus+PIV 1-3 was found in 1.1% (Table 1).

It is noted that IF tests or cell cultures are used in most of the studies investigating viruses as respiratory tract infection agents in our country rather than molecular methods. In a study performed by Yüksel et al. (12) in Manisa, respiratory tract viral pathogens were investigated by direct IF method in nasopharyngeal aspirate samples belonging to 151 children who were diagnosed with LRTI between 2002 and 2004. 25.2% of the samples were found to be positive in terms of respiratory tract viruses. In this study, RSV and adenovirus were reported to be the most common viruses with a rate of 31.5%. This was followed by parainfluenza with a rate of 26.3% and influenza with a rate of 23.6%.

Respiratory syncytial virus is a major cause of lower respiratory tract diseases especially in young children and it has been shown that children who had had RSV infection in infancy have wheezing attacks and asthma later (13,14,15). The rate of 61.2% we found in our study supports this information. Tanır et al. (16) reported the frequency of RSV to be 44.7% in children with lower respiratory tract symptoms. Yılmaz et al. (17) found RSV with a rate of 35% and 39% in nasopharyngeal secretions obtained from infants with acute bronchiolitis in a study conducted in two periods in İstanbul. In a another multi-center study performed by Kanra et al. (18) between 2000 and 2002 in Ankara, RSV was found with a rate of 29.5% in high-risk infants younger than 24 months hospitalized with findings of lower respiratory tract infections. We think our markedly high rate arised from the sensitivity of the method used compared with these studies. The studies in which lower rates were reported were performed using traditional methods. The insufficiency of traditional methods including cell culture and the tests directed to search antigen and antibody especially in sensitivity and speed has caused molecular methods to come into prominence.

There are considerably plenty of studies related with use of molecular methods in the diagnosis of respiratory viruses abroad. In a study performed by Wang et al. (19) in China, viral positivity was found with a rate of 59.5% in 489 nasopharyngeal samples obtained from children below the age of nine with LRTI using muliplex PCR. Respiratory scyntycial virus was reported to be the most commonly found pathogen with a rate of 19.4%. Do et al. (20) found 72% of the samples obtained from 309 children in Holland to be positive using multiplex PCR method and RSV was the most common agent with a rate of 24% (73 samples). In another study performed in 407 children below the age of five in Brazil using the same method, a viral pathogen was found in 85.5% of the samples and RSV was the most common virus with a rate of 37% (21).

The most common samples used in the molecular diagnosis of respiratory viruses were nasopharyngeal aspirates initially. However, nasopharyngeal aspiration is known to be an inconvenient method especially for young children because of the discomfort experienced during sampling. Currently, it is reported that samples obtained by noninvasive methods including especially nasal and throat swabs can be used with success in the diagnosis of respiratory viruses in studies in which different sampling methods are compared (22). Therefore, we preferred to use throat swab samples in our pediatric patient group in our study considering the conveniences of non-invasiveness, availability and good patient compliance during sampling.

The frequencies of viruses leading to lower respiratory tract infection show also variance according to seasons in infants and adults. Especially RSV has seasonal characteristic. It is the only virus characterized with causing to an outbreak in a certain period each year. Infections are observed frequently in the winter in warm climates and in rainy periods in tropical climates (23). The months during which viral infections were observed most commonly were found to be January, February and March also in our study with March observed to have the highest rate.

With introduction of use of molecular methods (especially multiplex PCR) in the diagnosis of respiratory viruses, studies in which multiple agents are found in the same patient have started to muliply in the literature. In our study, multiple agents were found in 7 samples (10.4%). These included RSV-rhinovirus association in 4 samples, RSV-coronavirus association in one sample and RSV-rhinovirus-coronavirus association in one sample. Respiratory syncytial virus was found to be the most common virus which caused to co-infection mainly in association with rhinoviruses. In the study performed by Frobert et al. (24) in children hospitalized in intensive care unit, the rate of co-infection was reported to be 35% and RSV was found to be the most common virus with a rate of 24.3% in co-infections. Again, Wang et al. found co-infection in 13.8% of the children with LRTI similar to our study group and HBoV, RSV, rhinovirus and adenovirus were also found with the highest rate, respectively in this group (19).

Conclusively, rapid and sensitive diagnosis of viruses leading to respiratory tract infections has currently become essential in terms of preventing unnecessary antibiotic usage and nasocomial infections which may be caused by these viruses. In addition, regional data reported from our region and from the world in vaccine development methods on which intensive studies are conducted will also be directive.

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Conflict of interest: None declared.

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