The investigation of pneumonia viruses by multiplex PCR in patients with pneumonia

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ABSTRACT
Viruses are the most common factors of pneumonia. The importance of quick diagnosis of viruses is gradually increasing. In this study, patients diagnosed with viral pneumonia, the causative viruses by multiplex PCR method, which aimed to show. Study between October 2010 and April 2011 of Pediatric Diseases and Chest Diseases Clinics invested or clinic for the diagnosis of pneumonia seen and treated, 300 patients were enrolled in. Nasopharyngeal swab samples were taken from the patients, and in these examples, viruses were detected by the multiplex PCR method. A total of 300 patients have been detected a virus on the 139 (46.3%). 114(38%) cases of virus detected patients are children and 25 (8.3%)cases are adults. The incidence of RSV pneumonia among all age groups were higher than the other viruses. In total, 44.6% RSV, influenza A 13.6, rhinovirus19.4%, parainfluenzavirus 6.5%, adenovirus 3.6%, influenza type B 4.3%, coronavirus 2.9%, human metapneumovirus 1.4% were found. Multiplex PCR tests, for respiratory infections is easy, fast and reliable tests. These tests must be located between the routine clinical microbiology laboratories, diagnostic tests.

Key words: Viral pneumonia, nasopharyngeal swab sample, multiplex PCR.

INTRODUCTION
Viral lower respiratory tract infections (LRTI) and their complications are among leading causes of morbidity and mortality in childhood and adulthood. Respiratory syncytial viruses (RSV), influenza viruses and parainfluenza viruses (PIV), adenoviruses, rhinoviruses and coronaviruses are often cited as the most frequently encountered causes of (LRTI). In recent years, with widespread use of nucleic acid-based techniques in the diagnosis of respiratory tract diseases of unknown causes, human metapneumovirus, new coronaviruses and human bocaviruses have been defined and it has been indicated that many of the respiratory tract diseases are caused by viruses. Although they vary depending on regions, age groups and the immune state of the host, the most frequently identified factors leading to the occurrence of (LRTI) are RSV, parainfluenza virus and rhinovirus in early childhood, whereas they are RSV and influenza viruses for older children and adults (1-3).

While 90 % of the pneumonias that occur during the first year of life are of viral origin, this ratio falls to 50 % during the school age. These data indicate that in our country pneumonias are an important health concern that causes high mortality and morbidity among children below age 5 (1,4,5).

In recent years, various polymerase chain reaction (PCR) techniques have been developed for the diagnosis of viral respiratory tract infections. One of these techniques, Multiplex PZR method, offers three features together, i.e. high sensitivity, shorter period of yielding results and being able to identify a few pathogenes in a single sample. Respiratory system viruses such as rhinovirus, coronavirus, and human metapneumovirus, whose existence had not been adequately substantiated through conventional methods, became definable thanks to Multiplex PZR (6,7).

This study aimed at determining disease-causing viruses in hospitalized patients or outpatients who were diagnosed with pneumonia through the multiplex PZR method and revealing incidences of viral pneumonia.
MATERIALS AND METHODS

Samples:
This study, which was intended to identify causes of viral respiratory tract diseases, included 300 patients (157 male and 143 female (girls/women), mean age 20.3) who applied to Pediatric Clinics and Clinics of Chest Diseases between October 2010 and April 2012 with symptoms of raile, rhonchus and fever and who had pneumatic infiltration associated with viral pneumonia on their chest radiography. The ages, genders and complaints of the patients in the study group, the duration of their complaints and whether they suffered from another disease or not were recorded in the patient information card.

Our study was approved by the Ethical Board of our faculty with the decision number 2010/022 dated April 4th, 2010 and supported by the Scientific Research Projects Coordination Office with project number 10102008.

Samples of nasopharyngeal swab were taken from the patients before any treatment was initiated. These samples were taken by the doctor by entering the nose with a swab and moving it two or three times on both tonsil medials and behind the uvula. The samples that were thus taken were carried to the laboratory using the eSwab Liquid Amies (Copan, Italya) transport medium. The samples were kept at -20°C until they were worked on.

The study was conducted in four stages:

Nucleic Acid Isolation: A commercial viral DNA/RNA extraction kit (Viral Gene Spin, Boca Scientific Inc., USA) was used for isolation procedures. 150 µl were taken from the nasopharyngeal swab material, which was vortexed and mixed, and the extraction procedure was performed in accordance with the suggestions of the manufacturer.

cDNA synthesis: cDNA was obtained using the reverse transcription kit (RevertAid First Strand cDNA synthesis kit, Fermentas) in accordance with the recommendations of the manufacturer.

PZR Amplification: The following regions were targeted: F gene for RSV A-B, segment 7 gene for influenza virus A and segment 1 gene for influenza virus B, HN gene for PIV 1-2-3, 5’NTR gene for rhinovirus, pol gene for adenovirus, S gene for coronavirus 229E/NL63, M gene for coronavirus OC43/HKU1 and F gene for human metapneumovirus. Simultaneous target amplification was performed for each virus using DPO (Dual Priming Oligonucleotide) primers as suggested by the manufacturer (Seeplex RV12 ACE Detection, Seegene, Korea).

Agarose gel electrophoresis: Following the loading of PZR products (5 µl PZR ürünü + 1 µl gel loading paint) and DNA Ladders (10 µl) to the gel, electrophoresis was implemented for 30 minutes at 180 volt and 50 mA. The results were compared with DNA Ladders (guides) and interpreted. If internal controls and markers had not formed, then the study was considered invalid and repeated.

RESULTS

Nasopharyngeal swab samples taken from 300 patients, of whom 160 were children and 140 were adults, were included in the study. 139 samples (46.3 %) were positive, of which 114 (38 %) were children whereas 25 (8.3 %) were adult patients. The role of viral causes was statistically more significant in the child patient group diagnosed with pneumonia than in the adult group (according to the t test, p> 0.463). While the highest number of cases was determined in the 0-4 age group, the highest ratio in terms of viral etiology was identified in the 5-9 age group. It was found that the number of cases and viral etiological effects decreased with age. The distribution of the results by age groups is given in Table 1.

While RSV was the most frequently identified virus in all age groups, rhinovirus ranked second in children. In adults, on the other hand, influenza type A was the most common cause together with RSV. The distribution of the viruses identified according to whether the patients were children or adults are given in Table 2.

DISCUSSION

Viral lower respiratory tract infections (LRTI) are the most common causes of morbidity in pediatric patients. Viral pneumonias may cause severe diseases and deaths especially in immunosuppressive patients or immunocompetent baby and old patients. Detection of respiratory viruses can be improved with molecular techniques. Multiplex PCR methods were developed with the aim of detecting many viruses simultaneously. More than one causative viruses can be investigated simultaneously in materials obtained from nasopharyngeal swab samples using the Multiplex
PZR method. Moreover, determination of viral causes that are hard to demonstrate through conventional methods or that have been rarely demonstrated so far can now be identified (8-10).

In recent years, search for tests yielding fast and reliable results that will facilitate the use of neuroaminidase inhibitors, which have been widely used in the prophylaxis and treatment of viral infections, in the early stages of the infection against the cause has gained significance (11,12).

Indeed, viral etiology was investigated in the samples that were taken from the patients who were diagnosed with viral pneumonia in our study through clinical markers and radiographic findings using the Multiplex PZR method.

In our study, the virus was determined in 32 samples (22.8%) from among nasopharyngeal swab samples taken from 140 adult patients. While the highest number of cases was determined in the 50-59 age group, the highest ratio in terms of viral etiology was found in the 18-29 age group.

RSV was the most commonly identified virus whereas influenza type A ranked second. They were followed by Rhinovirus and Influenza B.

In a study conducted outside of our country, i.e. in Korea, by Lee et al. in 2009 using the Multiplex PZR method, the virus was identified at a rate of 50% in 220 patients who were hospitalized in the pediatrics clinics due to prediagnosis of viral pneumonia and it was reported that it was 90% compatible with the viral culture. In the same study, RSV and influenza type A viruses were the most commonly identified causes RSV, rhinovirus, influenza type A virus, paramyxovirus virus and adenovirus have appeared to be the most frequently encountered causes in various studies (12-15). These are in parallel to the findings of our study. However, we are of the opinion that the diagnostic criteria for certain patient groups, distribution of seasonal causes or specific epidemic features of the season under study may change the ranking in different studies in terms of dominant viruses. Yet, we can say that certain viruses are predominantly responsible for viral diseases (16,17). The importance in etiology of causative agents such as rhinovirus that have begun to appear more frequently in routine test panels is becoming clearer, while it is a fact that causes such as human pneumoviruses, which have become more definable through various diagnostic methods especially PZR, have begun to come to the foreground in recently published reports (18-21). However, human pneumovirus has not been found in our study.

In our study, viruses were identified as causes of pneumonia in 32 patients out of 140 adult patients. The proportion of viral etiology was higher in studies conducted on children. It is an expected situation that with age, immunity will be acquired against viral infections in childhood and viral etiology will decrease in pneumonia (22). However, such low results for viral etiology in a population diagnosed with viral pneumonia can be attributed to the presence of other viruses which could be a pathogen but not included in our test panel, diagnostic interference by chronic diseases that increase by age, or our failure to exclude non-viral causes adequately.

RSV was the most frequently identified cause (37.5%) in the adult population of our study. While influenza type A ranked second at a rate of 28.1%, Rhinovirus and Influenza B came third at a rate of 12.5%. Although influenza viruses are causes that have the highest incidence for adult LRTI in many studies, RSV infections exhibit a relative increase due to the existence of influenza vaccines, rising social consciousness about vaccination and increasing demand for it (23).

It is observed that adenovirus, which is endemic all year round and can occur in all age groups, occupied a 6.25% percentage in our adult patient group. In a study conducted in our country, 5% positivity was found in a population consisting of soldiers, where incidence for this virus was expected to be high (24-26).

The fact that the coronavirus, which mostly causes upper respiratory tract infections, and other less frequent pathogens lead to LRTI is a subject that is associated with childhood, old age and immunosuppression (26-28). This situation is also valid for multiplex agents viral infections As a matter of fact, the data that we obtained are in support of this result because coronavirus and infections caused by multiplex viruses were not found in our study.

Screening of viral pathogens in nasopharyngeal sample using multiplex PZR is a fast, easy and reliable method which can yield results within 8 hours. Moreover, due to its contribution to the identification of some viruses, which are hard to grow in culture and whose effects have not be adequately determined until very recently, it is a good alternative to conventional methods. Although its high cost is considered a disadvantage, cost/benefit discussions should be made taking into account the importance of early diagnosis for patients in risk groups who have viral infections and can benefit from antiviral treatment.
Table 1. The distribution of results in variety age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Virus positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Child 0-4 (n:130)</td>
<td>92</td>
</tr>
<tr>
<td>Child 5-9 (n:20)</td>
<td>15</td>
</tr>
<tr>
<td>Child 10-15 (n:10)</td>
<td>7</td>
</tr>
<tr>
<td>Total Child (n:160)</td>
<td>114</td>
</tr>
<tr>
<td>Adult 16-29 (n:35)</td>
<td>16</td>
</tr>
<tr>
<td>Adult 30-39 (n:25)</td>
<td>6</td>
</tr>
<tr>
<td>Adult 40-49 (n:35)</td>
<td>2</td>
</tr>
<tr>
<td>Adult 50-59 (n:40)</td>
<td>1</td>
</tr>
<tr>
<td>Adult 60-70 (n:5)</td>
<td>0</td>
</tr>
<tr>
<td>Total Adult (n:140)</td>
<td>25</td>
</tr>
<tr>
<td>Total (n:300)</td>
<td>139</td>
</tr>
</tbody>
</table>

Table 2. The distribution of viruses

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Child % (n)</th>
<th>Adult % (n)</th>
<th>Total % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>38.8 (54)</td>
<td>5.8 (8)</td>
<td>44.6 (62)</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>18 (25)</td>
<td>1.4 (2)</td>
<td>19.4 (27)</td>
</tr>
<tr>
<td>Influenza type A</td>
<td>7.9 (11)</td>
<td>5.8 (8)</td>
<td>13.7 (19)</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>5.8 (8)</td>
<td>0.7 (1)</td>
<td>6.5 (9)</td>
</tr>
<tr>
<td>Influenza type B</td>
<td>1.4 (2)</td>
<td>2.9 (4)</td>
<td>4.3 (6)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>2.2 (3)</td>
<td>1.4 (2)</td>
<td>3.6 (5)</td>
</tr>
<tr>
<td>Coronavirus 229E/NL63</td>
<td>2.9 (4)</td>
<td>-</td>
<td>2.9 (4)</td>
</tr>
<tr>
<td>RSV + Rhinovirus</td>
<td>2.2 (3)</td>
<td>-</td>
<td>2.2 (3)</td>
</tr>
<tr>
<td>Metapneumovirus</td>
<td>1.4 (2)</td>
<td>-</td>
<td>1.4 (2)</td>
</tr>
<tr>
<td>RSV + Influenza type A</td>
<td>0.7 (1)</td>
<td>-</td>
<td>0.7 (1)</td>
</tr>
<tr>
<td>RSV + Adenovirus</td>
<td>0.7 (1)</td>
<td>-</td>
<td>0.7 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>82 (114)</td>
<td>18 (25)</td>
<td>100 (139)</td>
</tr>
</tbody>
</table>

\[X^2 = 42,8586\]

REFERENCES


