DETECTION OF HUMAN METAPNEUMOVIRUS IN NASOPHARYNGEAL ASPIRATES OF BRAZILIAN CHILDREN BY IMMUNOFLUORESCENCE ASSAY.

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Key words: human metapneumovirus; immunofluorescence assay; children; acute respiratory infections.
ABSTRACT

The circulation of human metapneumovirus is related to seasonal patterns of respiratory syncytial virus. The aim of this study was to determine the frequency of hMPV infection diagnosed by indirect immunofluorescence in children attending emergency department and general wards of Hospital Infantil Albert Sabin in Fortaleza, Ceará, northeastern Brazil, during the respiratory syncytial virus season in 2006. The analysis included 389 samples. Antigens of hPMV were detected in 39 samples,

INTRODUCTION

Human metapneumovirus (hMPV) is a causal agent of acute respiratory disease, mainly in children (Williams et al. 2006, Carr et al. 2005, Luchsinger et al. 2005, Kim et al. 2005, Crowe 2004, Dönnner et al. 2004, Boivin et al. 2003). Most studies on hMPV have used reverse transcriptase polymerase chain reaction (RT-PCR) due to the limited growth of this virus in cell culture, the unavailability of rapid antigen detection tests until 2005, and the lack of standard serological assays. Studies from different countries have shown the circulation patterns of the respiratory syncytial virus (RSV) and hMPV to overlap considerably and have revealed similarities in the clinical presentation of infections caused by these viruses (Al-Sonboli et al. 2006, Wolf et al. 2006, Cuevas et al. 2003, Greensill et al. 2003).

Rapid diagnostic methods, such as antigen detection by immunofluorescence, have been shown to be very useful in detection of respiratory viruses due to the short incubation periods and epidemic or even pandemic potential of these agents (Lipson 2002). This method has been used for hMPV detection directly in clinical samples in a small number of
studies (Ebihara et al. 2005, Percivalle et al. 2005, Fenwick et al. 2007, Manoha et al. 2008). The aim of this study was to determine the frequency of hMPV infection diagnosed by an indirect immunofluorescent-antibody test (IFA) in nasopharyngeal aspirates from children diagnosed with acute respiratory infection (ARI) at a pediatric hospital in Fortaleza (northeastern Brazil) during a RSV season in 2006.

METHODS

Local of study- The study was carried out at Hospital Infantil Albert Sabin (HIAS) in Fortaleza. The HIAS, the busiest public pediatric tertiary-level care facility in the state, has an average load of 8615 outpatient consultations, 4475 emergency patients and 613 admissions monthly. Fortaleza, the capital of the state of Ceará, northeastern Brazil, is a city of 2.3 million inhabitants, at sea level, 4° south of the Equator.

Study population- Infants, children and teenagers (0-16 years) with ARI attending the emergency department and general pediatric wards of HIAS were included in the study. Patients were included in the study if they had one or more of the following symptoms: cough, coryza, sore throat, earache, breathing difficulty, stridor, wheezing and fever (≥ 37.5°C) within seven days of onset. The children’s caretakers gave their written informed consent. The study was approved by the research Ethics Committee at the HIAS (resolution 196/2007).

Virological analysis- Nasopharyngeal secretions obtained by suction through a nasal catheter with a specimen trap were collected from each patient included in this study. Three slides were prepared from each sample. One slide of each sample was analyzed previously by IFA that was performed using the Respiratory Panel I Viral Screening and
Identification (Chemicon International, Temecula, CA, USA), following the manufacturer’s instructions, for detecting antigens of the following respiratory viruses: parainfluenza viruses 1, 2 and 3, RSV, influenza A and B, and adenovirus. The epidemic period of RSV in 2006 (March-July) was determined by review of results of IFA. Slides (stored at -80°C) of all samples collected during this period were retrospectively analyzed by IFA to detect hMPV as described previously (Ebihara et al. 2005). In summary, smears were covered with monoclonal antibody 1B7 (Chemicon International, Temecula, CA, USA) at a dilution of 1:80, and a fluorescein isothiocyanate conjugated rabbit anti-mouse IgG antibody (Chemicon International, Temecula, CA, USA) at a dilution of 1:40 with 0.001% Evan’s Blue. Both antibodies were incubated at 37°C for 30 minutes. Following incubation the slides were washed twice in PBS for 10 minutes and mounted with PBS-glycerin (1:1). Slides prepared with LLC-MK2 cells infected by hMPV, and the same cells not inoculated, were used as positive and negative controls of the IFA. These cells were kindly supplied by Dra. Divina Queiroz, Universidade Federal de Uberlândia, Minas Gerais, Brazil. Samples with at least 20 epithelial cells per field at X 400 magnification under a fluorescent microscope were considered of good quality for analysis. The presence of cytoplasm fluorescence in at least one cell was defined as a positive result.

RESULTS

A total of 653 samples were collected of children attended at the HIAS in 2006. The RSV season was observed to be from March to July 2006 when 389 samples were collected. In this period 103 samples were considered positive for at least one of the viruses tested in the first IFA (76 RSV, 23 influenza A, and 2 adenovirus, 1 parainfluenza 1, and 1
parainfluenza 3). A total of 286 samples were negative (73.6%) for the five viruses cited above and also to influenza B and parainfluenza 2. Figure 1 shows the monthly distribution of infections and co-infections by viruses analyzed in this study during the RSV season of 2006. The presence of hMPV was tested in all 389 samples previously tested to the other viruses. Antigens of hPMV were detected in 39 samples, three of which were from children co-infected with RSV. No co-infections with hMPV-other viruses were observed. Figure 2 shows hMPV-positive and negative samples of nasopharyngeal aspirates analyzed by IFA. hMPV was the second-most frequently detected virus after RSV in the period of study. Results of IFA analysis and the frequency of viruses researched are showed in Table 1.

**Figure 1.** Monthly distribution of infections by respiratory syncytial virus (RSV), human metapneumovirus (hMPV), influenza, adenovirus, parainfluenza 1 (PF1), parainfluenza 3 (PF3), and coinfections RSV/HMPV during the RSV season in 2006.
Table 1. Results of indirect immunofluorescence analysis of all samples collected during the respiratory syncytial virus season in 2006.

<table>
<thead>
<tr>
<th>Indirect immunofluorescence</th>
<th>Number</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples collected during respiratory syncytial virus season</td>
<td>389</td>
<td>100</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>76</td>
<td>19.5</td>
</tr>
<tr>
<td>Human metapneumovirus</td>
<td>39</td>
<td>10</td>
</tr>
<tr>
<td>Influenza A</td>
<td>23</td>
<td>6.0</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Parainfluenza virus 1</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Parainfluenza virus 3</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Figure 2. Indirect immunofluorescence staining of nasopharyngeal epithelial cells for hMPV antigens (A: IFA-positive staining to hMPV; B: sample negative to hPMV).
DISCUSSION

We retrospectively assessed the occurrence of hMPV infection in a pediatric population in Fortaleza during the RSV season of 2006. The finding of hMPV-positivity in 12.6% of samples screened negative (36/286) for seven different viruses supports recently published findings suggesting this agent may be responsible for a considerable number of cases of ARI of previously unidentified etiology (Cuevas et al. 2003, Greensill et al. 2003, Gerna et al. 2005, Al-Sonboli et al. 2006, Wolf et al. 2006, Manoha et al. 2007). hMPV has been detected in 5-16.2% of samples screening negative for several different viruses in a number of studies (Williams et al. 2006, Noyola et al. 2005, Debur et al. 2007, Órdas et al. 2006). In the present study a significant number of cases of ARI in children attending HIAS during the RSV season were diagnosed with hMPV. As shown by our data, during the period covered by the study, hMPV was more frequently detected than all other viruses (except RSV) analyzed.

Co-infections of hMPV and other respiratory viruses are common. The number of hMPV-RSV co-infections reported in this study lies within the wide range (0.5-70%) reported in the literature (Manoha et al. 2007, Al-Sonboli et al. 2006, Wolf et al. 2006, Gerna et al. 2005, Semple et al. 2005, Cuevas et al. 2003, Greensill et al. 2003). The high rates of co-infection with hMPV-RSV reported in some studies may be due to the overlapping of virus seasons (Al-Sonboli et al. 2006, Cuevas et al. 2003, Principi et al. 2006). No cases of hMPV-influenza A or other viruses analyzed were observed in this study, although co-infections of hMPV with other respiratory viruses had been reported in several studies (Heikkinen et al. 2008, Sarasini et al. 2006, Chano et al. 2005, Gerna et al. 2005, Manoha et al. 2007, Sasaki et al. 2005).
Most studies on hMPV employ molecular methods, such as RT-PCR, because they are less time-consuming and more sensitive than viral isolation (Madhi et al. 2007, Principi et al. 2006, Gray et al. 2006). However, the high cost of products and equipment required to perform RT-PCR is still a barrier to the routine use of this technology in laboratories around the world, many of which – like ours – employ immunofluorescence assays to identify respiratory viruses (respiratory syncytial virus, influenza A and B, adenovirus and parainfluenza 1, 2 and 3).

A diagnostic test should among other things be quick to perform in order to provide the physician with timely information when considering prevention or treatment measures. However, immunofluorescence assays are not only less costly than RT-PCR but also faster to perform: while RT-PCR requires approximately 6 hours, immunofluorescence takes from 20-30 minutes (direct immunofluorescence) to 60 minutes (indirect immunofluorescence).

With the advent of commercially available anti-hMPV monoclonal antibodies (MAbs), many studies have been published using IFA to directly detect hMPV in clinical samples (Ebihara et al. 2005, Percivalle et al. 2005, Fenwick et al. 2007, Manoha et al. 2008). The hMPV detection rate of the present study (10%) is higher than the rates obtained in other recently published studies (3.1% and 5%), but much lower than rates (25% and 45%) reported in early studies. A review of the IFA hMPV detection rates and sensitivities in those early studies revealed paradoxically higher hMPV detection rates and lower sensitivities, possibly accounted for by the selection criteria and the small sample size (Ebihara et al. 2005, Percivalle et al. 2005). In one study, 23 of 40 samples tested positive by previous RT-PCR profiling (Percivalle et al. 2005). In another study samples were
collected in April and May when hMPV is known to peak in the study area. More recently published studies, such as our own, have used larger samples collected continuously over longer periods (Ebihara et al. 2005).

Variations in IFA sensitivity for hMPV may be related to the use of monoclonal or polyclonal antibodies. Recently developed MAbs have enhanced IFA sensitivity, compared to IFA sensitivity rates (73.3% and 73.9%) reported in the early studies. In a study using anti-hMPV polyclonal antibodies, IFA and RT-PCR yielded similar sensitivities (Ingram et al. 2006). The use in IFA of recently developed MAbs yielded sensitivity (96.6%) very close to that reported for IFA using polyclonal antibodies (Fenwick et al. 2007). Results obtained with IIF and RT-PCR (100% sensitivity) were closely correlated in a study using MAbs which recognize the N protein. In spite of the high sensitivity achieved, only 3.1% out of 1386 samples tested positive for hMPV (Manoha et al. 2008).

In summary, IFA makes it possible to detect hMPV in a considerable number of cases of ARI of previously unknown etiology, and has shown hMPV to be the second-most frequently identified virus in infections investigated during a five-month period in 2006. To our knowledge, this is the first report using IFA to diagnose hMPV in children living in Brazil.

ACKNOWLEDGMENTS

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq- Grant: 620053/2004-6) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
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