Prevalence of Human metapneumovirus infection among patients with influenza-like illness: Report from a Tertiary Care Centre, Southern India

G Nandhini, *S Sujatha, N Jain, R Dhodapkar, K Tamilarasu, S Krishnamurthy, N Biswal

Abstract

Background: Human metapneumovirus (HMPV), discovered in the 21st century, has emerged as an important cause of influenza-like illness in children and adults causing mild upper respiratory tract infection to severe bronchiolitis and community-associated pneumonia. The aim of this study was to determine the prevalence of HMPV in the Union Territory of Puducherry, India, as part of National Influenza Surveillance Programme. Materials and Methods: From November 2011 to December 2013, a total of 447 nasopharyngeal samples were collected from patients with acute respiratory infections and tested for HMPV RNA by real-time polymerase chain reaction. Results: HMPV was identified in 23/447 (5%) samples with 11/23 in the age group of 14–30 years. Most of the HMPV infections were mild with no fatalities. Two patients were co-infected with the respiratory syncytial virus and one with influenza B virus. The seasonal distribution showed increasing HMPV infection cases in rainy months except for a peak in summer of 2012. Phylogenetic analysis based on the sequences of the nucleoprotein gene of one HMPV strain showed a high degree of sequence identity with Indian strains obtained during 2006 and 2011. Conclusion: This study shows that HMPV infection is more common in adults than in children. Sequence homology suggests the circulation of closely related HMPV strains within the country.

Key words: Acute respiratory infections, Human metapneumovirus, India, influenza-like illness, real-time polymerase chain reaction

Introduction

Human metapneumovirus (HMPV), a virus discovered in the 21st century, has emerged as the second most common cause of influenza-like illness (ILI) next only to respiratory syncytial virus (RSV) in children.[1] The discovery of HMPV was delayed due to its poor growth and long incubation cycle in a restricted number of cell lines[2] and it was identified only after the introduction of molecular techniques in the diagnosis of respiratory infections. Currently, there is no recognized gold standard method to detect HMPV although real-time polymerase chain reaction (RT-PCR) is most often employed.

Initial reports suggested that HMPV was responsible for ILI only in children, but subsequently it was also shown to cause infections in adults ranging from mild upper respiratory tract infection to severe bronchiolitis and community-associated pneumonia.[3-4] Like other respiratory viruses, HMPV follows a seasonal pattern of distribution that varies from one geographic location to another and depends to a large extent on the climatic conditions prevalent in the area.

In India, the first report of HMPV was documented by Rao et al., in 2004 from Pune[5] followed by others from Lucknow, Vellore, Hyderabad and New Delhi with most of these studies targeting children as the study population.[6-8] There are very few studies in India showing the prevalence of HMPV in adults and children. Hence, in 2009, a regional influenza surveillance centre was established in our hospital with the objective of identifying viruses causing ILI in both adults and children.

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adults and children the Union Territory of Puducherry, India. The objective of this study was to determine the prevalence of HMPV and seasonal distribution of HMPV infection from Union Territory of Puducherry, Southern India.

Materials and Methods

Geographic details and climatic conditions of the target area

The Union Territory of Puducherry lies at a latitude of 11°46′–12°30′ North and a longitude of 79°36′–79°52′ East in the Southern part of India. The territory is situated on the Coromandel Coast of Bay of Bengal and it extends over an area of 479 km². The total population is 12,44,464 as per the 2011 census. This territory has a hot and humid climate. Summer is from March to July and winter is from December to February. The rainy months of this area are September to December. Meteorological (temperature and rainfall) data for Puducherry for the period between November 2011 and December 2013 was obtained from the Regional Meteorological Centre, Chennai (No. 8043/CS-[ER]-032 dated 30-07-2014).

Study design

As part of the ongoing National Influenza Surveillance Programme, nasopharyngeal samples were collected from patients (both children and adults) attending the outpatient care facilities three sentinel centres from Union Territory of Puducherry; Jawaharlal Institute of Postgraduate Medical Education and Research Hospital, Karaikal Government Hospital and Pondicherry Government General Hospital, with symptoms of ILI, defined as fever (≥38°C) plus either a cough or a sore throat. In addition, specimens were also collected from hospitalized patients who satisfied the case definition of severe acute respiratory illness. Patients with a history of acute respiratory infection in the preceding 30 days were excluded. For this study, we included patients who were recruited during the period November 2011 - December 2013 and had their residence in Union Territory of Puducherry. The study protocol was approved by our Institute Ethics Committee (Human studies). written informed consent was obtained from all the patients or their guardians as appropriate, before specimen collection. A detailed questionnaire was used to collect the following information: Age, sex, area of residence, medical co-morbidities, influenza vaccination status, clinical symptoms, day of illness, travel history, contact history and pregnancy status (where required). Meteorological (temperature and rainfall) data for Puducherry for the period between November 2011 and December 2013 was obtained from the Regional Meteorological Centre, Chennai (No. 8043/CS-[ER]-032 dated 30-07-2014).

Specimen processing

Nasopharyngeal swabs were transported in 3 ml viral transport media (Hi-Media, Mumbai, India) on ice, stored at −80°C and RNA was extracted using a viral RNA kit (QIamp, Qiagen, CA, USA) and RT-PCR for HMPV detection was performed within a week of collecting the samples.

Real time polymerase chain reaction assay

Reverse transcriptase RT-PCR (Step one real-time system, Applied Biosystems, USA) was performed with AgPath-IDTM One Step RT-PCR kit (Applied Biosystems, CA, USA) according to the manufacturer’s instructions. Primers and TaqMan probes (Applied Biosystems, CA, USA) used were specific to the N-gene segments of HMPV. The quality of specimen collected was checked by testing the samples for RNase P (internal control). Positive and negative controls were included for all the amplifications.

Sequencing

Sequencing was done using ABI 3730 Genetic analyser (Applied Biosystems, USA) using gene-specific forward and reverse primers of the nucleoprotein gene. To determine the lineages and to evaluate genetic diversity, 26 HMPV N-gene sequences submitted from different countries, available in the GenBank database were retrieved. The sequences were aligned, and a dendrogram was generated by the neighbour-joining method with the MEGA6 software. Sequences of the HMPV isolates have been deposited in GenBank under the accession number KM281807.

Statistical analysis

Comparison of data based on gender, admission status and the need for mechanical ventilation was analysed by performing Fisher’s exact/Chi-square test. Mean age (years) in different groups was analysed using Student’s t-test. Statistical significance was concluded if the P < 0.05. Data were analysed using online statistical software, QuickCalcs (GraphPad Software).

Results

From November 2011 to December 2013, a total of 447 nasopharyngeal samples were collected from patients with acute respiratory infections. HMPV was identified in 23/447 (5%) samples by RT-PCR assay. Out of 23 positives, 16 were female and 7 were male, with a male:female ratio of 1:2.3 (P < 0.05). Among the positives, 65% (15/23) were adults and 35% (8/23) children [Table 1]. The gender-wise distribution and age wise distribution shows double the positivity in females compared to males and in adults compared to children. Eleven of the 23 positive patients were in the age group of
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14–30 years, with one positive case found in the older adult group (≥51 years) [Table 1].

Among the HMPV positives (23), 61% (14) were from ILI patients and 39% (9) were admitted due to severe respiratory illness and 26% (6) were on mechanical ventilation, although there were no fatalities [Table 2]. The major clinical symptoms observed in HMPV positive patients were fever (100%, 23/23), cough and sore throat (>80%, 21/23 and 19/23). About 17% (4/23) had episodes of wheezing and 9% had a history of asthma. Two patients were co‑infected with RSV and one patient was co‑infected with influenza B virus. There was no co‑infection of HMPV with influenza A/H1N1 or influenza A/H3N2 in this study (data not shown). Nearly 65% (15/23) of patients with HMPV infection gave a history of close contact with a case of respiratory illness in the 1–2 weeks preceding their illness while 17% (4/23) had travelled outside Puducherry in the week preceding the symptom onset.

In 2011, 15% (5/34) of the samples were HMPV positives, in 2012, 5% (14/296) and in 2013, 3% (4/117) [Figure 1]. The maximum number of HMPV positive cases 12, (52%) was observed in April-May 2012, when there was also a peak in the number of ILI cases recorded due to influenza H1N1 outbreak in Puducherry. Of the remaining 11 HMPV positive cases, ten were observed during the rainy season of all the years under study [Figures 2 and 3].

The phylogenetic tree comparing the sequence of a representative HMPV strain from our study isolates (GenBank number #KM281807) with other published sequences in GenBank is shown in Figure 4. The HMPV strain obtained in our study was similar to many Indian strains which was circulated during 2006 and 2011 (New Delhi, Pune and Vellore) and a few global strains (Peru, Netherlands, Taiwan). The strains mainly from

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### Table 1: Demographic details of HMPV positive and negative patients; represented as number (%)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=447)</th>
<th>HMPV positives (n=23) (5%)</th>
<th>HMPV negatives (n=424) (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, range (years)</td>
<td>19.76, 0.08-83</td>
<td>20.34, 0.08-63Si</td>
<td>19.73, 0.08-83</td>
</tr>
<tr>
<td>Age groups, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>209 (47)</td>
<td>8 (35)</td>
<td>201 (47)</td>
</tr>
<tr>
<td>Adults</td>
<td>238 (53)</td>
<td>15 (65)</td>
<td>223 (53)</td>
</tr>
<tr>
<td>Age, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5 years</td>
<td>173 (39)</td>
<td>5 (22)</td>
<td>168 (40)</td>
</tr>
<tr>
<td>6-13 years</td>
<td>36 (8)</td>
<td>3 (13)</td>
<td>33 (8)</td>
</tr>
<tr>
<td>14-30 years</td>
<td>123 (27)</td>
<td>11 (48)</td>
<td>112 (26)</td>
</tr>
<tr>
<td>31-50 years</td>
<td>74 (17)</td>
<td>3 (13)</td>
<td>71 (17)</td>
</tr>
<tr>
<td>≥51 years</td>
<td>41 (9)</td>
<td>1 (4)</td>
<td>40 (9)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>215 (48)</td>
<td>16 (70)*</td>
<td>199 (47)</td>
</tr>
<tr>
<td>Male</td>
<td>232 (52)</td>
<td>7 (30)</td>
<td>225 (53)</td>
</tr>
</tbody>
</table>

Si Students t-test was used to analyse the data. P>0.05. *Fisher’s exact test was used to analyse the data. P<0.05. RR=2.466 (95% CI=1.35-5.8). CI=Confidence interval, HMPV=Human metapneumovirus, RR=Relative risk

### Table 2: Clinical characteristics of HMPV positive and negative patients; represented as n (%).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=447)</th>
<th>HMPV positives (n=23) (5%)</th>
<th>HMPV negatives (n=424) (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outpatients (ILI)</td>
<td>296 (66)</td>
<td>14 (61)*</td>
<td>282 (67)</td>
</tr>
<tr>
<td>Inpatients (SARI)</td>
<td>151 (34)</td>
<td>9 (39)</td>
<td>142 (33)</td>
</tr>
<tr>
<td>Need for mechanical ventilation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical symptoms, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>447 (100)</td>
<td>23 (100)</td>
<td>424 (100)</td>
</tr>
<tr>
<td>Cough</td>
<td>421 (94)</td>
<td>21 (91)</td>
<td>400 (94)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>388 (87)</td>
<td>19 (83)</td>
<td>369 (87)</td>
</tr>
<tr>
<td>Chills and rigors</td>
<td>206 (46)</td>
<td>13 (57)</td>
<td>193 (46)</td>
</tr>
<tr>
<td>Wheezing</td>
<td>72 (16)</td>
<td>4 (17)</td>
<td>68 (16)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>64 (14)</td>
<td>3 (13)</td>
<td>61 (14)</td>
</tr>
<tr>
<td>Earache</td>
<td>8 (2)</td>
<td>1 (4)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>GI symptoms</td>
<td>27 (6)</td>
<td>1 (4)</td>
<td>26 (6)</td>
</tr>
<tr>
<td>Co-morbidity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>44 (10)</td>
<td>3 (13)</td>
<td>41 (9)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>7 (2)</td>
<td>0</td>
<td>7 (1)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>7 (2)</td>
<td>1 (4)</td>
<td>6 (1)</td>
</tr>
<tr>
<td>Cancer</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asthma</td>
<td>13 (3)</td>
<td>2 (9)</td>
<td>11 (3)</td>
</tr>
<tr>
<td>Transplant</td>
<td>5 (1)</td>
<td>0</td>
<td>5 (1)</td>
</tr>
</tbody>
</table>

*Chi-square test was used to analyse the data. P=0.65,
**Chi-square test was used to analyse the data. P=0.13.
ILI=Influenza-like illness, SARI=Severe acute respiratory illness, HMPV=Human metapneumovirus, GI=Gastrointestinal

**Figure 1:** Total number of influenza-like illness samples and Human metapneumovirus positives in different years of the study period

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Argentina, Canada and Brazil were grouped separately, indicating low homology with the studied strain.

**Discussion**

The HMPV prevalence of 5% (23/447) in the present study was comparable to most other reports from India\textsuperscript{[11‑13]} although considerably lower than the 12% reported from Vellore in South India and New Delhi in North India.\textsuperscript{[7,8]} Prevalence rates vary widely across the globe from as low as 1.7% in Cambodia\textsuperscript{[14]} and 2.6% in the USA\textsuperscript{[15]} to as high as 27% in Taiwan.\textsuperscript{[16]} These differences could reflect the type of study population; methods employed and the epidemiological situation in which the studies were carried out, outbreak versus routine surveillance.

**Figure 2:** Human metapneumovirus positivity in 2011–2012 along with monthly mean maximum temperature (C) and total rainfall (mm) data

**Figure 3:** Human metapneumovirus positivity in 2013 along with monthly mean maximum temperature (C) and total rainfall (mm) data

**Figure 4:** Human metapneumovirus phylogenetic tree - nucleoprotein gene of one representative sample was amplified, sequenced, and compared to 27 other published sequences from GenBank. We have labelled the sequences as "accession number/virus/country or state/date of collection". Nucleotide sequences are aligned, and phylogenetic analyses were performed using the neighbour-joining method (MEGA6 software) (●Study strain, ▲Indian strains)
In the present study, most of the HMPV positive cases belonged to the 14–30 years age group (11/23) while only 5/23 of the cases were in children <5 years of age which is contradictory to the recent reports from North and Eastern India.[12,13] In the initial period of discovery, HMPV was thought to be an agent of ILI only in children.[2] Subsequent reports showed that HMPV is also common in adults and can cause severe respiratory disease in the elderly leading to hospitalization.[11,15] The reason for these differences in the age distribution of HMPV is unknown but may be due to the varying levels of exposure and immunity.

The male:female ratio in our study was 1:2. Similar female preponderance was observed in reports from Egypt[17] and China.[18] However, there is a contradictory report from Latin America, where in 2011, they observed no difference in the prevalence among the sexes.[19]

The clinical characteristics of our patients with HMPV infection were similar to those described in previous reports[12,20,21] with mild respiratory symptoms including fever, cough and sore throat. Unlike RSV, mortality is rare in HMPV infections, which was corroborated in the present study. However, HMPV is considered to be an important cause of wheezing[12] and this association was reflected in our study with nearly 17% (4/23) of the HMPV infected patients reporting wheezing as a clinical symptom. This virus has also been associated with other lung pathologies like asthma and Chronic Obstructive Pulmonary Disease (COPD). Although we did not observe COPD in our group of patients, 9% (2/23) of the positive patients had bronchial asthma suggesting a possible association of HMPV bronchiolitis with asthma exacerbations. A similar association has been made in other studies relating asthma and HMPV[22,23] although there are also contradictory reports of a lack of such an association.[24]

Viral co-infections are relatively common, but their significance and effect of clinical severity is a subject of ongoing debate. In the present study, we encountered three such patients with co-infections, two with RSV and 1 with influenza B (data not shown). Conflicting reports exist in the literature about the association of such dual infections with clinical severity, some studies showing the increased risk of severe infections[23,24] while others documented no such association.[25,28] In our study, the 2 cases where HMPV was found along with RSV presented with severe infection requiring hospitalization.

Studying the seasonality of respiratory viruses becomes important as on occasion it can be used to predict the etiological agent. Seasonal trends of respiratory viruses differ between tropical and temperate regions of the world. Unlike temperate regions where respiratory infections are common during winter months, tropical regions see more than one period of virus activity. In a large country like India, climatic conditions are very different from the Northern and Southern regions. All the HMPV reports from North India showed increased activity during the winter months, that is, lower temperature (November-March).[8,11] Similar observation was made in the present study; except for a peak in HMPV cases in summer of 2012 (May) where we experienced a concomitant outbreak of influenza A/H1N1 in this region, although there were no co-infection cases. In the subsequent year, there were no cases of influenza A/H1N1 (data not shown) and very few cases of HMPV, whether this association is significant or not, requires a longer period of study.

Phylogenetic analysis based on the sequences of the nucleoprotein gene in an HMPV strain showed a high degree of sequence identity with Indian strains obtained during 2006 and 2011. This homology within sequences obtained from different Indian states suggests circulation of closely related strains within the country. The strain also clustered closely with a few global strains showing the possible transmission of HMPV strains between countries due to international travel and transport.

Sequencing of a larger number of strains would have given us more information on the profile of HMPV strains circulating in Puducherry, although this could not be done due to technical constraints. Although beyond the scope of this study, detection of other viral and bacterial agents of ILI would have given a more comprehensive picture of aetiology of this condition.

To conclude, the results of this study indicate a higher prevalence of HMPV in adults than in children, with most infections being mild, although its possible association with bronchial asthma needs further investigation. The climatic pattern of HMPV infection in Union Territory of Puducherry closely resembled that in other parts of the country with the exception of a possible peak that occurred in the summer of 2012.

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Integrated Disease surveillance programme (IDSP) and National centre for Disease control (NCDC), New Delhi, India.

Conflicts of interest

There are no conflicts of interest.

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