THE HIGHEST PREVALENCE OF HUMAN METAPNEUMOVIRUS IN AHwAZ CHILDREN ACCOMPANIED BY ACUTE RESPIRATORY INFECTIONS

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Abstract

Purpose: The newly described human metapneumovirus (hMPV) has been recently discovered as an etiological agent of acute respiratory infections (ARTI) in infants and children. The aim of this study was to determine the prevalence of hMPV and its potential role as causative agent of ARTI in Ahwaz children. Methods: In the present study, we examined 124 nasal swabs from infants affected by ARTI for the presence of hMPV by RT-PCR technique. Results: Sixty-eight out of 124 (54.4%) cases were positive for hMPV which is the highest incidence of hMPV ever reported in the world, 94.1% of positive cases belonged to genotype A; whereas no B genotype was detected. Our positive hMPV children were affected by upper (URTI) as well as lower respiratory tract infection (LRTI); however, LARTIs had higher prevalence. Conclusions: We suggest a probable role of F protein alteration as the causative agent for the highest prevalence of hMPV infection among Ahvaz children.

Key words: Ahwaz, acute respiratory infections, human metapneumovirus
Finally, to rule out the presence of hRSV, the most frequent respiratory viral agent responsible for ARTI, a nested RT-PCR was carried out on hMPV positive samples as previously described.\[12\]

**Results**

hMPV prevalence among male (48.5%) and female (51.5%) patients was almost similar. A further analysis by two-tailed independent *T*-test showed that there was no correlation (*P*-value > 0.05) between gender in children less than two years and the rate of infection due to hMPV. The age of hMPV positive children was between 10 days and 24 months and the average age was 17 months. Age distribution of hMPV positive children is reported in Fig. 1.

Sixty eight out of 124 (54.4%) samples were positive for Hmpv. Among positive samples 94.1% belonged to Genotype A (Fig. 2), whereas no genotype B was detected; 5.9% of positive cases remained untypable. 33.8% (23/68) of hMPV positive children were co-infected by hRSV.

An interesting observation in PCR results with hMPV common primer was that an extra band of 192-184 bp could be observed beside the main band of 281 bp, but not in positive control which was obtained from an Italian isolate. The extra band could not be eliminated modifying the PCR conditions such as lowering the MgCl2 solution concentration and/or increasing the annealing temperature (Fig. 2). To ensure that this extra band was not a chromosomal spurious band we made an extra experiment with whole blood extracted nucleic acid as template and no extra band was detected.

A comparative correlation of hMPV infection frequency with clinical manifestation in ARTI is reported in the table. Statistical analysis did not demonstrate any significant correlation between each clinical manifestation and the positivity for hMPV: \( \chi^2 \) test *P*-value = 0.140. Further analysis showed a significant difference between the rate of hMPV infection in LRTI (pneumonia, bronchiolitis and bronchitis) versus URTI in favor of LRTI. Signs and Symptoms associated with the presence of hMPV are reported in the table.

Seventy-four percent (50/68) of positive cases were less than 12 months old. Statistical analysis showed that there was no significant linear correlation (Pearson Correlation test *P*-value = 0.065) between an increase in age and hMPV infection rate; although the rate of hMPV infection showed a strong correlation in the 0-12 months age group (\( \chi^2 \) *P*-value < 0.001), versus the 12-24 months age group.

HMPV infection rate was 54.2% in fall and 56.4% in winter, with almost no statistical difference. In addition, our analysis revealed no significant correlations between the rate of infection and meteorological indices: amount of precipitation (*P* = 0.5) number of days with thunder storm or dust (*P* > 0.6), air temperature (*P* = 0.508), air pressure (*P* = 0.795) and relative humidity (*P* = 0.64).

**Discussion**

As previously described, the prevalence of hMPV in children population is between 1.5 and 43%, but the prevalence of hMPV among Ahwaz children was the highest so far reported in the world (54.2-56.4%).\[2-5,8,12,13\]

According to other reports,\[5\] our results confirmed that wheezing and cough were in order the most prevalent sign and symptom in hMPV positive children affected by ARTI (Table). In addition, our study showed that bronchiolitis was the most significant clinical diagnosis associated with the presence of hMPV: 28% compared to 67% reported in other studies.\[4\]

Despite the fact that in our study hMPV cover a broad rang of URTI and LRTI, we found that hMPV was involved in LTRI rather than UTRI.\[2-5,8,12\]
The rate of hMPV infected children was inversely proportional to their age: a decrease in rate of infection was associated with an increase in age. This finding is in concordance with the fact that by the age of five months almost all of the children had encountered hMPV.\textsuperscript{[12]}

The reasons for high prevalence of hMPV among Ahwaz children could be either viral internal factors like the efficiency of viral replication, the ability of the virus to escape from host immune responses, the route of transmission or environmental factors that are not totally independent from the former factors and include geographical position, climate situation, seasonal fluctuation, yearly variation and finally genetic susceptibility of patients.\textsuperscript{[2,13]} Moreover, in a definite geographical region, factors like the patient age, their health, the rate of circulating antibodies and the genetic characteristic of the population can be determinant factors.

Previous studies have shown that there are three major surface antigenic determinants in hMPV called G, SH and F glycoproteins.\textsuperscript{[1,2]} Although hMPV G and SH glycoproteins are the most capable for the genetic heterogeneity of hMPV, only immunity against F protein is considered to be significantly neutralizing or protective.\textsuperscript{[14]} Further studies have shown that some alteration in the F gene, may confer a host immunological evasion to the virus without having an interaction with viral growth or virulence.\textsuperscript{[15]} As earlier mentioned, the result of RT-PCR experiments in this study with the hMPV common pair primers resulted in an extra band which is equal to an extra recognition site inside the F gene. Since an extra recognition site could not be created unless a sequence alteration carried out, this phenomenon raises the hypothesis that an alteration that happens in F gene led to a genetic heterogeneity accounting for host immune evasion. However, further investigations are needed to prove this hypothesis.

Another hypothesis for the probable role of immune evasion in high prevalence of hMPV in Ahwaz could be the age of the children involved. It has been shown in previous studies throughout the world that children less than six months had fewer infections than children between 6 and 12 months.\textsuperscript{[2-5,13]} In our study the age of infection was under six months and also in children belonging to the two groups of 0-6 months and 6-12 months the infection rate was the same. Since it is generally believed that maternal immunity is capable of conferring immunity against most of infectious agents in babies less than six months, a decrease in infection age could be an indication that maternal immunity did not work well against a potential new variant of hMPV.

As mentioned before, the geographical distribution of hMPV infection in children less than 6 months is variable throughout the world. Because Ahwaz climate is similar to a tropical region it is comparable with a South American country like Brazil (17%) even if Ahwaz is situated in the northern hemisphere.\textsuperscript{[2]} HMPV prevalence in Ahwaz is three times higher than in Brazil. On the other hand our data are very close to that reported from Italy (43%).\textsuperscript{[8]} So the climate contribution in the prevalence of hMPV might not be the major factor influencing the high prevalence of this virus in Ahwaz city.

Yearly genetic variation may be responsible for viral evasion from pre-existing immune response due to the circulation of another genotype.\textsuperscript{[8]} Since we carried out our experiment during one year we could not estimate the effect of the above factor on hMPV prevalence.

Many studies have confirmed the role of seasonal fluctuation in hMPV prevalence.\textsuperscript{[3]} As previously mentioned, no significant difference was observed between fall and winter in hMPV prevalence in this study.

Another factor that may contribute to the high hMPV incidence is the genetic susceptibility of the population. Although no studies have yet analysed the effect of this factor on the prevalence of hMPV, some previous studies revealed the influence of genetic susceptibility on the incidence of other human paramyxovirus infections.\textsuperscript{[16]}

Since we did not rule out all of respiratory viral agents in our hMPV positive children we can not conclude that hMPV was the sole causative agent of all ARTI in this study. However, our results showed that RSV, which has been confirmed to be the major causative agent of ARTI in children less than five years, coinfected only 1/3 of

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**Table: Distribution of signs, symptoms and clinical diagnosis in children with human metapneumovirus infection**

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Symptoms</th>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia 9 (13.2%)</td>
<td>Cough</td>
<td>Wheezing</td>
</tr>
<tr>
<td>Bronchiolitis 19 (27.9%)</td>
<td>Coryza</td>
<td>Rales</td>
</tr>
<tr>
<td>Bronchitis 14 (20.6%)</td>
<td>Fever</td>
<td>Rhinitis</td>
</tr>
<tr>
<td>Common cold 14 (20.6%)</td>
<td>Irritability</td>
<td>Pharyngitis</td>
</tr>
<tr>
<td>Laryngitis 8 (11.8%)</td>
<td>Wheezing</td>
<td>(Abnormal tympanic pain)</td>
</tr>
<tr>
<td>Croup (laryngo)</td>
<td>Anorexia</td>
<td>Rales</td>
</tr>
<tr>
<td>tracheo bronchitis 4 (5.9%)</td>
<td>Vomiting</td>
<td>Diarrhoea</td>
</tr>
</tbody>
</table>

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our hMPV positive cases. This data raises the probable role of hMPV as an important causative agent of ARTI. Considering all the factors that may contribute to the high prevalence of hMPV infection among Ahwaz children, we suggest a role of F protein alteration as the most probable causative agent.

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References


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