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Prevalence of Human Metapneumovirus (hMPV) in Children with Wheezing in Shiraz-Iran

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ABSTRACT

Human *metapneumovirus* (hMPV) infection plays an important role in the pediatric respiratory infections. The aim of this study was to determine the relationship between asthma and wheezing with hMPV in hospitalized children.

Nasal pharyngeal swabs obtained from 120 children aged 1-60 months, hospitalized during a one year period, were tested for the hMPV by RT-PCR. HMPV was detected in 20 (16.6%) of patients suffering from wheezing. Some patients in addition to wheezing had asthma 10.8%. This infection occurred predominantly from October 2008 to September 2009.

Key words: Asthma; Iran; Metapneumovirus; Pediatrics

INTRODUCTION

In 2001, Van den Hoogen reported the isolation of a novel virus in young children with respiratory infections.[1] This virus was classified in the *metapneumovirus* genus of the sub-family pneumovirus, family paramyxoviridae of the order mononegavirales and given the provisional name of human *metapneumovirus* (hMPV).[2-7] hMPV has been identified in upper and lower respiratory tract infections in children and as causative agent of viral bronchiolitis in infants.[8-14] , It has been reported to cause disease similar to respiratory syncytial virus (RSV), with signs and symptoms ranging from severe cough, fever, rhinorrhea and influenza like symptoms to bronchiolitis, pneumonia and attack of wheezing. These features resemble those of RSV, which makes diagnosis of hMPV and RSV by clinical symptoms difficult.[3, 15] hMPV is second only to RSV detected by the incidence rate among infants and children.[10] hMPV has been isolated from respiratory secretions of all ages patients.[15] hMPV is prevalent mainly during winter and spring and has been isolated in different countries world-wide.[3,16,17]

In this study, we sought to investigate the role of hMPV in hospitalized wheezing children.

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MATERIALS AND METHODS

During 2008 fall/winter and spring seasons nasal discharge samples from 120 children less than 5 years old affected by acute wheezing and asthmatic patients constituted those who had already been labeled by a physician, suffering a second attack of wheezing or born to asthmatic patients were collected using nasal

swabs. Each swab was expressed in a micro tube containing 200µL phosphate buffer salin (PBS). Samples were finally stored at -70°C until use. All samples were thawed at 25°C and viral RNA was extracted with Roche pure viral nucleic acid kit and converted to cDNA by using Qiagen Sensiscript Reverse Transcriptase. The samples were then screened for hMPV by carrying out a PCR reaction with the common pair primer targeted regions of hMPV V and F genes that was conserved among Dutch, Australian, and Connecticut strains. The forward primers, 5-GCGCGTTCTGAGGACAGGTTGG and reverse primers, 5-GCGCTCAAGCCGGATGGTTTTGG, produced an amplicon that corresponds to nt 111-392 of the hMPV F gene (Gen Bank accession number AF371367). Concentration of PCR reagents were as follows: 0.5µM, MgCL2 1.5mM, dNTP Mix 0.2mM, and Taq polymerase 1.25U.

The first reaction was performed at 95°C for 15 min; then 35° cycles for 1 min. 94°C, (45-55)°C for 1 min, 72°C for 1 min; 72°C for 10 min as previously described. Negative samples for common primer were checked for the accurate performance of extraction and RT steps by carrying out a PCR reaction on RT products for 18s rRNA.[18]

RESULTS

Human *metapneumovirus* was detected in 20 (16.6%) of patients suffering from wheezing. some patients in addition to wheezing had asthma 10.8%.

A comparative study showed the frequency of hMPV infection to be correlated with clinical sign of bronchiolitis. hMPV accounted for exacerbation of pneumonia and asthma. Statistical analysis demonstrated significant correlation between clinical Manifestations and the positivity for hMPV (p=0.027). Further analysis showed also a significant correlation Between the rate of hMPV infection and bronchiolitis (56.3%). Signs and symptoms associated with hMPV infection are presented in table 1.

Table 1. Distribution of sign and symptoms in children affected by hMPV

Signs and symptoms	Percent
Fever	12%
Cough	100%
Runny nose	50%
Wheezing	100%
Rales	35.3%
Sneezing	58.8%
Asthma	10.8%
Bronchiolitis	100%

hMPV prevalence among male (75.5%) was almost three times greater than female (23.5%) patients (p< 0.05). The age of hMPV positive children were between 1 to 60 months and the average age was 16 months.Age distribution of hMPV positive children is shown in figure 1. Of positive cases 14.3% were

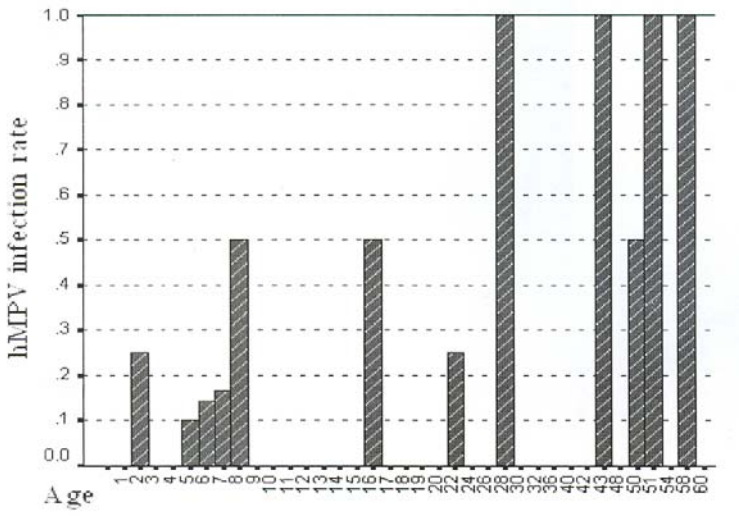

Figure 1. Age distribution of hMPV infection in children

hospitalized less than 4 days. Statistical analysis showed that there was a significant correlation between an hMPV infection and hospitalization of more than 2 days.

There was no significant linear correlation between asthma history in patient's family and hMPV infection rate (Fisher exact test P=0.39). In addition our analysis revealed no significant correlations between the rate of hMPV infection and cyanosis (P =0.4).

The infection was prevalent during October 2008 to September 2009.

DISCUSSION

We detected hMPV in 20 of 120 admitted patients (16.6%) with acute wheezing. Our results suggest hMPV is an important viral cause of acute wheezing in hospitalized children in Shiraz, Iran. Other studies reported the prevalence of positive-hMPV patients in acute wheezing as 10%,[4] 13%[5] (13.3%,[2] 4%,[18] and 8.9%[6] and in other respiratory tract infections as 54.4% in acute respiratory infections,[19] 4% in bronchiolitis,[13] 3.5% in bronchiolitis and pneumonia[10] and 5.5% in respiratory disease.[14] Prevalence in Arabpour, et al report in Ahwaz (Iran) is not consistent with other studies and even with findings of the present study.[20]

Seasonal distribution of hMPV revealed increasing prevalence in winter and spring. This result is in agreement with those of other studies,[3,15,17] but Piergangeli et al, showed one peak in winter and another in June Geographic variation may explain the difference.[10]

The prevalence in males was three times greater than females. Although in others studies, significant differences were not detected.[9,3]

The most common clinical findings in our patients with hMPV infection were wheezing and cough. Fever was found in 12% of patients. High fever with extended duration was the most frequent sign in hMPV infection compared with that of RSV.[19] Other presentations were hoarseness without strider, febrile seizure, truncal rash which was blanchable, non pruritic, maculopopular and transient diarrhea, congested pharynx, enlarged liver, otitis media, acute exacerbations of asthma, bronchiolitis, pneumonia, lymphopenia and elevated transaminases.[14]

Our study revealed no significant correlation between hMPV and cyanosis, respiratory rate and degree of hypoxemia.

In our study, the patients with hMPV tended to have a longer hospital stay more than 2 days, in accord with Pieris M, et al.[19] report. Also hMPV appeared to have a longer duration of fever and hospital stay than RSV and influenza virus infection.[14]

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