

Research Article

Prevalence of human metapneumovirus in children with acute respiratory tract infection Shiraz, Iran during 2014-2015

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ABSTRACT

Background: Human metapneumovirus (HMPV) is a newly discovered viral agent of respiratory infections. In present study we determined prevalence of HMPV in children less than 10 years with acute respiratory infections (ARTI) that referred to the health centers of Shiraz University of Medical Sciences, Iran.

Methods: Nasal swabs were obtained from 180 children less than 10 years old with respiratory tract symptoms during fall, winter and spring seasons of 2014 and 2015. The specimens were tested for HMPV F gene using one step RT-PCR.

Results: HMPV was detected in 30 (16.66%) of 180 children with (ARTI) less than 10 years old. Approximately 63.33% of HMPV positive patients aged less than 3 years and 86.33% were younger than 5 years old. The clinical symptoms were bronchiolitis, wheezing and cough.

Conclusions: The frequency distribution of HMPV revealed that most patients aged between 1 to 5 years and this virus is one of the respiratory viral agents to causes ARTI, especially children less than 5 years old.

Keywords: HMPV, Shiraz, Iran, Sub-genotype

INTRODUCTION

Human metapneumovirus (HMPV), was first discovered in the Netherlands by Den Hoogen and colleagues in 2001, and isolated from a pediatric patient having symptoms similar to those of HRSV infection. These viruses were classified in the metapneumovirus genus of the sub-family pneumovirinae, family paramyxoviridae of the order mononegavirales and given the provisional name of Human metapneumovirus (HMPV).^{1,2}

Nowadays, two genotypes of HMPV have been detected based on genetic analysis of all genes of HMPV. These are A and B genotypes which are divided into sub-genotypes A1, A2, and B1, and B2, respectively. The sub-genotype A2 is divided further into sub-genotypes A2a and A2b.³⁻⁵ The clinical manifestations of HMPV

infection are indistinguishable from those of Human respiratory syncytial virus (HRSV) infection, especially in young children. HMPV patients are generally diagnosed with bronchiolitis, bronchitis, and pneumonia.

They show common symptoms like fever, cough, hypoxia, upper respiratory tract infection, lower respiratory tract infection, and wheezing, however, the most common causes of hospitalization are bronchiolitis and pneumonia.^{6,7}

Since 2001, HMPV has been detected in 4–16% of patients with ARTIs. The annual incidence of HMPV may vary in the same area.² Outbreaks occur often in the spring and winter months, mostly January to March in the northern hemisphere and June to July in the southern hemisphere.^{8,9}

Objective

The goal of this study was to determine the prevalence, Age distribution and clinical characteristics of HMPV infection and its potential role as causative agent of ARTI in Shiraz children.

METHODS

Throat swabs were collected from 180 children less than 10 years of age with ARTI referred to the health centers of Shiraz University of Medical Sciences in the fall/winter and spring seasons from 2014 to 2015.

RNA extraction

Each swab was expressed in a micro tube containing 200µL phosphate buffer saline (PBS). Samples were finally stored at -70°C until used. Viral RNA was then extracted using Roche extract viral nucleic acid kit, Germany.

Pathogen screening

HMPV detected by One Step PCR (TUF) reaction with specific primers targeting regions of F genes including HMPV-FF-347 (GAGCAAATTGAAAATCCCAGACA) and HMPV-FR-347 (QGAAACTGCCGCACAACATTTAG), the regions conserved among Dutch, Australian, and

Connecticut strains. The 50 µl of PCR mix comprised RT-PCR Master mix (2X) 25 µl, forward primer 1µl (10µM), reverse primer 1µl (10µM), template RNA 1 µl, add D.W up to 50 µl. The cycling conditions in eppendorfs mastercycler were as follows: 50°C for 30min cDNA synthesis ,the PCR protocol was 95°C for 15 min one cycle; followed 35 cycles at 95°C for 45sec ,55°C for 30 sec , 72°C for 90 sec; and finally 72°C for 5 min.

Statistical analysis

Statistical analysis of the results was performed using SPSS (Statistical Package for Social Sciences) program version 18. Mean, standard deviation, and percentage were used as descriptive statistics. Categorical variables were analyzed by chi-square test, and P values ≤0.05 was considered significant.

RESULTS

Demographic characteristics

A total of 180 nasopharyngeal swabs were collected from inpatients and outpatients with ARTI and less than 10 years-old between January 2014 to February 2015. The patients were divided into 3 groups according to the age 84 (47%) were less than 3 years old, 3 to 5 were 51 (28%) and 8 to 10 were 45 (25%). Of these, 104 (57.8%) were males with median age of 2.3±1 years and 76 (42.2%) females with median age of 4±1 years.

Table 1: Frequency of clinical manifestations in HMPV positive patients.

Clinical manifestation	Gender	Number	Percent	Total	
				Number	Percent
Bronchiolitis	Female	5	55.55%	9	30%
	Male	4	44.44%		
Fever	Female	16	53.33%	30	100%
	Male	14	46.66%		
Cough	Female	8	66.66%	12	40%
	Male	4	33.33%		
Myalgia	Female	9	60%	15	50%
	Male	6	40%		
Wheezing	Female	16	53.33%	30	100%
	Male	14	46.66%		

Viral prevalence

The HMPV was detected in 30 (16.66%) of 180 samples. These specimens were acquired from 30 patients of whom 16 (53.33%) were males and 14 (46.66%) females. A further statistical analysis revealed that there was no correlation between gender and HMPV rate infection (P-value=0.077). PCR results for some samples are shown in (Figure 1).

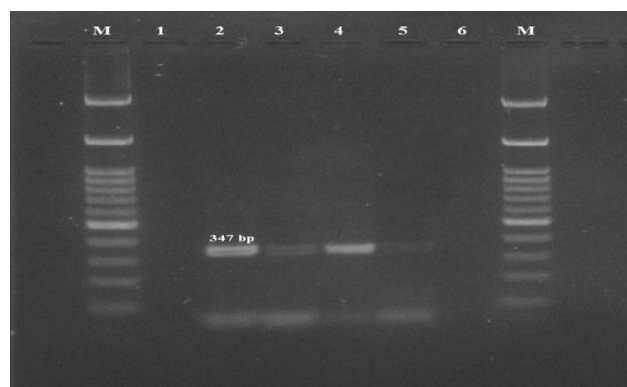
Age distribution and HMPV infection

To precisely analyze the age distribution of HMPV-positive patients were grouped by age in years as follows: <3, 3-5, 5-10 years .Approximately 19 (63.33%) of HMPV positive patients aged less than 3 years and 26 (86.33%) were younger than 5 years-old and 4 (13.33%) were 5 to 10 years of age. The frequency distribution of HMPV infection revealed that most patients aged;

between 1 to 5 years. Statistical analysis about correlation between an increase in age and HMPV infection rate demonstrated that there was no significant linear correlation (Pearson Correlation test P -value=0.195).

Seasonal distribution

About seasonal distribution present study showed that prevalence of HMPV infection in winter was higher than other seasons {22 (73.33%) and 8 (26.66%) of positive samples were belonged to winter and fall, respectively}. Statistical analysis about difference between fall and winter in HMPV prevalence demonstrated there was significant correlation between season and HMPV rate infection ($p < 0.05$).



M: Ladder (100bp); 1: negative control; 2: positive control; 3: number 23; 4: number 54; 5: number 78; 6: negative sample.

Figure 1: PCR result obtained from some positive and negative samples.

Clinical characteristics of HMPV infection

The information on the clinical characteristics of HMPV infection was collected by trained attending physicians, using questionnaires filled in by the patients referred to Health centers of Shiraz University of Medical Sciences. Accordingly, all HMPV positive patients had fever and wheezing, 12 (40%) had cough, 9 (30%) had bronchiolitis and 15 (50%) exhibited Myalgia (Table 1).

DISCUSSION

Present study found that frequency of HMPV was 16.66% in children with ARTI in Shiraz. Our findings demonstrated HMPV is an important viral causative agent of ARTI in Shiraz children. Limited studies conducted in Iran up to now reported the prevalence rate of HMPV infection to be about 1-17% in Shiraz and Tehran.

The results of this study were consistent with 16.6% prevalence rate of HMPV reported previously by studies in Shiraz, Iran, whereas results of one study in Ahwaz during 2005 were unexpected they showed 54.4% of samples were positive for HMPV that is not consistent

with Tehran and other studies in the different part of world and even with results of the this study.¹⁰⁻¹²

Several studies reported different frequencies of HMPV in hospitalized children in various parts of the world over the 5 last years. The prevalence of HMPV have been reported from Brazil 2014 (8.3%), China 2013 (2.24%), Taiwan during 2013-2014 (32.7%), Kuwait 2010-2013 (5.3%), Mexico 2012-2013 (22%) and Malaysia 2010-2012 (1.1%).^{8,11,13-18}

As mentioned before, prevalence of HMPV in our study was 16.66%, but annually studies in this area about HMPV infection rate may report different rates because the annual incidence of HMPV may vary in a given location.²

Present study showed that in regard to the age about 86.66% of patients aged less than 5 years, a finding consistent with the results of other studies performed in different parts of the world since 2001, which highlights the importance of HMPV infection in this age group.^{11,15,19-21}

Globally a vast majority of respiratory tract infections (RTIs) are caused by respiratory viruses including HMPV, which affect all age groups particularly young children aged less than 3 years, elderly, immunocompromised and hospitalized patients.^{10,11}

According to other studies, present findings confirmed that wheezing, cough and fever were the most prevalent sign and clinical manifestation in HMPV positive patients (Table 1).^{12,22,23} HMPV infections seem to have similar clinical symptoms as those of RSV infections and molecular methods are commonly used to differentiate between these viral infections.²¹ As for seasonal distribution, the higher HMPV prevalence is found in cold seasons especially winter.^{11,24,25}

CONCLUSION

In conclusion, this study showed 16.66% of samples were positive for HMPV infection that our findings emphasis on HMPV rules as causative agent of ARTI in Shiraz children, especially children less than 5 years old. Further studies are needed to exactly determine the frequency, peak of HMPV season in this region and HMPV pathogenesis in order to develop an effective vaccine.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee (IR.sums.rec.1394.s18).

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