

ORIGINAL ARTICLE

Detection of the Emerging Human Metapneumovirus and Its Comparison with the Respiratory Syncytial Virus among Children with Acute Respiratory Tract Infections

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ABSTRACT

Key words:

RSV/ hMPV/ acute respiratory infections/PCR

Background: Respiratory syncytial virus and human metapneumovirus are respiratory pathogens that circulate worldwide. RSV is the most common cause of respiratory tract disease in children; where children ≤ 3 years of age are usually affected. **Objectives:** The aim of the present study was to determine the frequency of hMPV and RSV infections among children with acute respiratory tract infection and to compare their occurrence in children suffering from acute respiratory tract infection. **Methodology:** This was a cross sectional study carried out on 50 patients diagnosed as having acute respiratory infections. The included patients were children up to 6 years of age selected from hospitalized patients in Abo El-Reesh hospital. Throat swabs were obtained. The collected specimens were subjected to viral RNA extraction and tested for the presence of hMPV and RSV by real time PCR. **Results:** RSV was detected in 4 out of 50 (8%) of the studied cases, while hMPV virus was not detected among our studied patients. All detected cases were less than two years old (median age 6 months) with a statistically significant association between detection of the RSV and age of patients ($P < 0.05$). There was no significant difference regarding the gender and the detection of the virus. **Conclusion:** Although hMPV is known as a cause of ARTI, it was not detected among our studied patients. RSV was detected in 8% of pediatric patients aged less than two years with ARTI.

INTRODUCTION

Acute of viral respiratory tract infections are a leading cause of illness in both children and adults. Most such infections are confined to the upper respiratory tract. However in infants and children, upper respiratory tract infections may spread to the lower respiratory tract, where they can cause more severe infections and even death¹. Human respiratory syncytial virus (RSV) is one of the common causes of respiratory tract disease in children; where children ≤ 3 years of age are usually affected.

It is an enveloped, non segmented, negative-sense RNA virus classified in the subfamily *Pneumovirinae* of the family *Paramyxoviridae*². In June 2001, researchers in Netherlands reported the discovery of a virus associated with respiratory tract disease in infants and children. Genetic studies of this newly discovered viral pathogen revealed that it was a paramyxovirus, and was named human metapneumovirus (hMPV)³. Symptoms of hMPV infection range from mild upper respiratory tract infections to severe lower respiratory tract infections⁴.

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Previous studies have isolated the virus from nasopharyngeal aspirates/swabs and throat swabs^{1,5,6}. Real time PCR has been described for hMPV, allowing amplification and quantitation of this pathogen in clinical samples being more sensitive, specific and rapid in detecting this virus than conventional reverse transcriptase PCR⁷.

METHODOLOGY

1- Study Design and subjects

The current study was carried out on 50 patients between May 2012 and May 2013. The included patients were children of both sexes under 6 years of age presenting with symptoms and signs of acute respiratory tract infection. The selected patients were children patients admitted to Inpatient Ward of Abo El-Reesh Pediatric Hospital, Cairo University with no history of chronic chest conditions, cardiovascular, gastrointestinal or neurological diseases. The study was approved by the ethical committee of Faculty of Medicine Cairo University and all patients' parents granted their consent to share in the study. 60% of the studied patients were in the age group of more than 2 years up to 6 years of age followed by the age group of

1-2 years representing 10% of total number of patients. The percentage of male patients was 42% while the percentage of female patients was 58%.

2- Sample collection and processing

A dry sterile throat swab was used to swab both the tonsils and the posterior pharynx. Then it was placed in a 15 ml sterile tube containing 2ml phosphate buffered saline (PBS) (Immco Diagnostics, USA) and labeled with the patient's ID. The applicator stick was then cut off. The swabs inside the 15 ml tubes were agitated vigorously for 10 seconds using a vortex mixer to free cells from the swab tip, then the swab were removed from the tubes and discarded followed by storing the samples immediately at -70°C until further analysis.

3- Molecular detection of RSV and hMPV

Viral RNA was extracted using QIAamp viral RNA mini kit (QIAGEN, Germany) according to the manufacturer's protocol, The kit combines the selective binding properties of a silicagel-based membrane with the speed of microspin. 140 μl of thawed sample was used for extraction by QIAamp Viral RNA spin protocol in a fully automated process on the QIAcube machine (QIAGEN, Germany). The extracted RNA was checked for concentration and quality using the NanoDrop 2000/2000c spectrophotometer. Quantitative real time PCR of RSV and hMPV was done according to the manufacturer's protocol in a one-step approach using Primer design, genesig advanced kit for Respiratory Syncytial Virus (all species)/Human Metapneumoviruses genomes (United Kingdom), the kits targeting the amplification of the nucleoprotein gene of both viruses. Detection was done by Applied Biosystem 7500 RT-PCR machine, USA.

4- Statistical analysis

Data were statistically described in terms of frequencies (number of cases) and percentages. IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used for data analysis. The probability of error at 0.05 was considered significant, while at 0.01 and 0.001 were considered highly significant.

RESULTS

Among the studied patients, the most common respiratory infection among all patients was acute bronchitis representing 34% followed by acute pharyngitis (22%), bronchopneumonia (20%), pneumonia (14%) and then bronchopneumonia and RD (8%) (table 1). HMPV was not detected in any of the studied patients while RSV was detected in 8% of all studied patients. The RSV positive cases were detected in the winter months (November 2012, December 2012 and January 2013).

There was no statistically significant relation between sex of patients and detection of the virus. As regards age of patients there was a statistically significant association between the age of the patients and the detection of RSV (table 2) with a median age of 6 months in the positive cases. Also there was a high statistical significant association between patients suffering from bronchopneumonia associated with respiratory distress and the detection of RSV (table 3). Moreover, there was no statistically significant association between duration of symptoms and the detection of the virus in studied patients (table 4).

Table 1: Types of respiratory tract infection

Type of infection	Number	Percent
Bronchopneumonia	10	20%
Pneumonia	7	14%
Acute bronchitis	17	34%
Acute pharyngitis	11	22%
Bronchopneumonia & RD	4	8%
Acute bronchiolitis & RD	1	2%
Total	50	100%

Table 2: Relation between age, sex of patients and detection of RSV

Variable	RSV detection		P value
	Detected	Not detected	
Age			
1-3 mon	2(50%)	1(2.2%)	<0.05
>3-6 mon	0	2(4.3%)	
>6-9 mon	0	4(8.7%)	
>9-12 mon	2(50%)	4(8.7%)	
> 1year-2years	0	5(10.8%)	
>2 years-6 years	0	30(65.2%)	
Sex			
Female	1(25%)	20(43.5%)	>0.05
Male	3(75%)	26(56.5%)	

Table 3: Relation between type of chest infection and the detection of RSV

Type of infection	Detected RSV no.(percent to total detected)	Undetected RSV (percent to total undetected)	P value
	n=4	n=46	
Bronchopneumonia	1(25%)	9(19.5%)	> 0.05
Pneumonia	1(25%)	6(13.04%)	> 0.05
Acute bronchitis	0	17(36.9%)	> 0.05
Acute pharyngitis	0	11(23.9%)	> 0.05
Bronchopneumonia & RD	2(50%)	2(4.35%)	< 0.01
Acute bronchiolitis & RD	0	1(2.17%)	> 0.05
Total	4(100%)	46(100%)	

Highly significant = P value < 0.01

Table 4: Relation between duration of symptoms (days) and detection of RSV

Variable	RSV detection		P value
	Detected	Not detected	
Duration of symptoms			
Min	3	2	
Max	5	7	>0.05
Median	4	5	

DISCUSSION

Viral etiology plays a significant role in ARTIs in infants and children. Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are two of the most frequent respiratory pathogens that circulate worldwide⁷. Among our studied patients, human metapneumovirus was not detected as a cause of ARTI. Our results are in agreement with a study in Upper Egypt on 520 patients from the period from 2005 till 2008, where hMPV was not detected among 300 of their patients in the first two years of the study, whereas in 2007 and 2008, hMPV was detected in only 4% of cases⁸. This study explained that the difference in the detection rate of the virus was due to variation in frequency of hMPV from year to year in a given region. A study in Brazil reported that among 217 pediatric patients, hMPV was responsible for 24% of cases of bronchiolitis in the 1st year (2002) but was not found among 106 studied patients in the 2nd year (2003)⁹. As a result of that yearly variation, the pattern of hMPV circulation remains unclear. On the other hand, slightly higher detection rates were reported by others. HMPV was detected in 1.1% in out of 350 hospitalized cases¹⁰. HMPV was only detected in 1.2% out of 167 hospitalized children suffering from acute respiratory tract infection¹¹.

The present study showed that RSV was detected in 8%. Our detection rate was consistent with a study over 13,982 children during the period from 2008 till 2011 in which RSV was detected as a cause of ARTI in 8.1%¹². Also, other study detected RSV in 8.5% out of 130 cases during 2007-2008 seasons¹³. The overall frequency for RSV infection among 100 children younger than 5 years of age with LRTI was 9%¹⁴. Our detection rate was in partial agreement with a study

from Japan who reported that the RSV detection occurred in 10% out of a total number of 8,163 children¹⁵. Another study reported that during the period from November 2006 till October 2009, RSV was detected in 13.5% out of a total number of 7220 patients¹⁶. A lower detection rate could be detected in other studies. RSV infection was detected in 1.7% out of 6,986 pediatric patients in the period from 1997 till 1999¹⁷. Detection rate for RSV infection was 3% of the studied cases in a study in Upper Egypt¹⁸. Out of 129 cases, RSV was positive in 5.4% of patients in other study¹⁹. Also a study reported a detection rate of 5.7% out of 316 children with ARTI²⁰. Moreover a study found that 5.7% out of 160 pediatric patients were infected with RSV²¹. On the other hand, a higher detection rate was reported in a number of studies. In a study on 486 respiratory samples collected from children aged less than 9 years old, RSV was detected in 24.7% of studied patients²². In another study from China, reported that 31.6% out of 1,387 were positive cases of RSV in the period from April 2006 till March 2009²³. The high detection rates for RSV reported by some of the previously mentioned studies might be attributed to the young patient age where some studies were conducted on patients less than one year old. It is to be noted that the present study included pediatric patients up to 6 years of age.

Our study showed a statistically significant association between patients' age and detection of the virus. All positive RSV cases were less than 2 years old, with a median age of 6 months in the detected cases, this is in agreement with a study which reported that RSV was rarely detected in children older than 3 years of age (1%) whereas, RSV was detected among 40% of infants younger than 3 months, 30% of those aged 3 to 6 months, 20% of infants aged between 6 & 12 months

and 10 % of those aged 1 to 3 years²⁴. Our results are also in agreement with a study on 2492 samples of children reported that there is a high statistically significant relation between the age of patients and the detection of the virus²⁵.

In the present study, there was no statistically significant relation between duration of symptoms and detection of RSV, this result coincides with another study showed that there was no significant correlation between detection of RSV and duration of symptoms among a total of 1,288 children younger than 2 years of age admitted to hospital with ALRTI²⁶. Also other study reported that the frequency of virus isolation in culture was 86% in children with less than 2 days duration of symptoms and 42 to 69 % in those with duration of symptoms of 2 days or more. However, this difference was not statistically significant²⁷.

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