

Detection of *Respiratory Syncytial Virus* in Hospitalized Children With Acute Lower Respiratory Tract Infections, Using RT PCR in Ahvaz, Iran

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ABSTRACT

Background: Acute respiratory tract infections (ARTIs) play a major role in hospitalizations of children, and *Respiratory syncytial virus* (RSV) is well recognized as the most important pathogen causing ARTI.

Objectives: This study aimed to evaluate the epidemiological and clinical patterns of RSV infection in children hospitalized for lower ARTI in Ahvaz, Iran.

Patient and Methods: Respiratory specimens collected from 100 children with lower ARTI from October 2008 until the end of April 2009, were screened for RSV using reverse transcription-polymerase chain reaction (RT-PCR).

Results: During the study period, 9 children had a positive result for RSV infection. The median age of these patients was 10 months. Bronchiolitis was the clinical diagnosis of patients with RSV infections. All of these patients were less than 24 months. Cough (77.7%) and chest wall retraction (100%) were the leading symptoms and signs respectively.

Conclusions: This study indicates that RSV is a substantial cause of respiratory tract infection in infants less than 2 years old. RT-PCR offers a rapid method for detection of RSV in hospitalized children with LRTI.

Keywords: Child; Respiratory Tract Infection; Respiratory Syncytial Viruses; Reverse Transcription Polymerase Chain Reaction

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1. Background

Acute respiratory tract infections (ARTIs) are a leading cause of hospitalization and mortality in children less than 5 years of age and represent a major health problem in the world (1). Viruses, including *Respiratory syncytial virus* (RSV), *Metapneumovirus*, *Influenza A* and *B*, *Parainfluenza* and *Adenovirus* are the most common etiological

agents for childhood acute respiratory tract illnesses (2). Among viral causes of acute respiratory tract infections, RSV has a significant role. Human *Respiratory syncytial virus* is an enveloped, single-stranded; negative-sense RNA virus from the genus *Pneumovirus*. The peak incidence of the RSV infections is between the second and sixth month of age. It can cause significant morbidity from upper re-

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This paper detects the rate of RSV in children with lower respiratory tract infections.

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spiratory infections, acute bronchiolitis, and bronchopneumonia to apnea in children (3, 4).

In hospitalized children, RSV infections occur at greater frequencies than other viral infections of the lower respiratory tract. It is identified as the etiologic agent in 60-90% of patients with bronchiolitis and in 25-50% of those with pneumonia (5, 6). In temperate countries, RSV outbreaks have a defined seasonality, occurring mainly during fall and winter while in tropical and semi-tropical countries, it mainly peaks during the rainy season (7, 8).

In addition to conventional viral culture techniques and serology, a recently described innovative, polymerase chain reaction (PCR) for the diagnosis of respiratory viral infections has also been shown to be useful because it offers an enhanced sensitivity combined with rapid detection. Even if certain viruses such as RSV can be grown in cell cultures, this method is not completely reliable and many scientists have begun to use reverse transcription polymerase chain reaction (RT-PCR) to identify infections (9, 10).

Information on the epidemiology of RSV infections in developing countries are still limited. Most of these studies have not used highly sensitive molecular techniques such as RT-PCR (11-13).

2. Objectives

The purpose of this study was to provide data from the detection of RSV by RT-PCR in hospitalized children up to the age of five years in Ahvaz, Iran.

3. Patients and Methods

The study population consisted of children less than 5 years of age, admitted to the Aboozar children's hospital with lower respiratory tract infections (LRTIs). It was designed to enroll subjects from the beginning of October 2008 until the end of April 2009. Newborn infants less than 28 days were excluded.

LRTIs were categorized as pneumonia or bronchiolitis, on the basis of clinical and roentgenographic findings. Bronchiolitis was defined as an acute respiratory illness characterized by rhinorrhea, cough, and dyspnea and diffuses wheezing, with peribronchial thickening and hyperexpansion on chest radiograph if available. Pneumonia was defined as dyspnea in a patient with focal rales or decreased breathing sounds and the presence of a focal infiltrate and/or consolidation on chest radiograph.

During hospitalization, the children's signs and symptoms were obtained by a review of medical charts. This information was reviewed and entered into a database. Informed consent was obtained from the parents.

Following the clinical assessment, nasopharyngeal specimens were collected by gently rubbing the deep nasal turbinate with Dacron swabs. They were kept in viral transport medium and were stored at -70° until further

testing was performed by the virology laboratory of the infectious diseases research center. Samples were obtained within 24 hours after admission.

3.1. RT-PCR

Initially RSV RNA was extracted from nasal samples by using high pure RNA nucleic acid kit (Roche company), followed by cDNA preparation for each sample. The Nested RT-PCR was carried out and the following primers were used: (Johnson)

G1- CCA TTC TGG CAA TGA TAA TCT C

G2- GTT TTT TGT TTG GTA TTC TTT TGC GA

G3- CGG CAA ACC ACA AAG TCA CAC

G4- GGG TAC AAA GTT AAA CAC TTC

The primers G1 and G2 were used for the first round. 25 µL of PCR master mix containing 5 µL of the cDNA from each sample, 12.5 of the 1 × PCR master mix, 50 pmol of each primer (G1 and G2) were added to the master mix. The PCR was performed for 40 cycles in the Techne Thermocycler UK, initially for 5 min at 95°C and finally for 5 min at 72°C for one cycle. The primers G3 and G4 were used for the second round. The 25 µL of PCR master mix containing 5 µL of cDNA from each sample, 12.5 of the 1 × PCR master mix, 50 pmol of each of the G3 and G4 primers were added to the master mix. The PCR was performed for 35 cycles, initially for 5 min at 95°C followed by, 1 min at 72°C, 1 min at 95°C and finally 5 min at 72°C for one cycle. The expected final PCR product was 326 bp.

This descriptive study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences.

4. Results

One hundred specimens from hospitalized children with LRTI were tested for possible RSV infection. There were 57 males (57%) and 43 (43%) females. The median age of patients at the time of study was 21 months and 63 patients (63%) were younger than 2 years (Table 1).

The clinical diagnoses at the time of admission and discharge were as follows: pneumonia 64 (64%) and bronchiolitis 36 (36%). The overall frequency for RSV infection among the 100 children younger than 5 years of ages admitted to Aboozar children hospital was 9%. The median age of RSV-infected children was 8.5 months. Bronchiolitis was the clinical diagnosis of patients with RSV infection. Six patients with RSV infection were in 1-12 months age group and 3 of them were in 1-2 years.

In children with RSV infection, cough was the leading symptom (77.7%) followed by coryza (66.6 %) and fever (55.5%). Evidence of lower respiratory tract infection were chest wall retraction (100%), wheezing (88.8%) and cyanosis (22.2%) (Table 2). The clinical outcomes for children in-

ected with RSV were good in general.

Table 1. Distribution of Patients With LRTI According to Age and Clinical Diagnosis

Age	Bronchiolitis No. (%)	Pneumonia No. (%)	Total No. (%)
1-12, mo	23 (23)	10 (10)	33 (33)
1-2, y	10 (10)	20 (20)	30 (30)
2-3, y	2 (2)	14 (14)	16 (16)
3-4, y	1 (1)	12 (12)	13 (13)
4-5, y	0	8 (8)	8 (8)
Total	36 (36)	64 (64)	100 (100)

Table 2. Symptoms and Signs Observed in Patients With RSV Infection

Symptoms and sign	Patients, No.	Percent
Cough	7	77.7
Coryza	6	66.6
Fever	5	55.5
Tachypnea	9	100
Wheezing	8	88.8
Cyanosis	2	22.2

5. Discussion

Viral infections are considered to be the most important cause of lower respiratory tract infections (LRTI). It is responsible for significant mortality and morbidity in children. RSV is one of the most frequent etiological agents causing LRTI, especially among young infants (14, 15).

RSV epidemics occur yearly but may alternate in occurrence between midwinter and early spring. Its distribution varies in different countries and seasons (7).

During this study RSV was detected in 9% of all included subjects. The percentage of laboratory-confirmed RSV cases in Greece, Jordan and Bulgaria were 5.4%, 12.5% and 8.46% respectively, that are comparable with our study (1, 16-18). The prevalence of RSV in this study is relatively low compared with data reported in northern European and American countries (10, 14, 15, 19). Other studies from Iran have showed incidences of RSV infection to be 12.9%, 15%, 16.8 % in cases of LRTI and all of these reports are from temperate regions (20-22).

The difference in RSV epidemiology in the world may be related to differences in climate conditions, environmental factors and severity of its epidemics from one year to another (10). Research has reported negative correlations between higher monthly mean temperatures and RSV infection in tropical regions (21). Ahvaz is located in the southwest of Iran and has a temperate weather during late fall-winter and a warm to hot weather dur-

ing the rest of the year not favoring the survival and the spreading of RSV.

Studies have shown that some pediatric patients with acute lower respiratory tract infection become simultaneously infected with multiple respiratory viruses. Dan peng et al. suggest that there is a high occurrence of multipathogen infections in children admitted with acute respiratory tract infections and that coinfection is associated with certain pathogens. In this study RSV was detected in only 5.7% of cases (23).

In addition, Arabpour et al reported a high prevalence (54.4%) of Human *Metapneumovirus* (hMPV), among Ahvazian children with respiratory tract infections. This study showed that hMPV was probably an important causative agent of acute respiratory tract infections in this area (24).

In our survey, there was a slightly higher incidence of RSV infection in males (1.3:1). This result was consistent with other studies. Sangar et al. suggested that being female was protective against RSV hospitalization (25, 26). In this study all of the children that were tested positive for RSV had bronchiolitis and the highest number of positive samples of RSV was from patients less than 1 year old. This tendency towards the younger age, in RSV infections has been reported by other studies (27, 28).

The clinical features of children with RSV positive samples observed in our study were similar to those of previous reports (14, 15). There were some limitations in our study. One of our limitations was that although more than 100 children presented the criteria for inclusion in this study, samples from the first 100 patients were collected, due to the limit established by processing and storage of samples. The second limitation was that we did not check out all of the respiratory viral agents in our patients. It seems that in our geographic position and climate situation the other viruses like hMPV have an important role in LRTI of children. The other limitation was that accurate differentiation between viral and bacterial pneumonia is not possible according to clinical manifestations and CXR findings, and some of our cases with negative PCR for RSV may have bacterial pneumonia.

Infections with respiratory viruses are a common cause of morbidity and mortality around the world. A better understanding of the epidemiology of respiratory viral infections may be used for timely, specific antiviral therapy, prophylaxis, and vaccination. Future prospective surveillance over an extended period in this region is needed to accurately identify the epidemiology of viruses.

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Authors' Contribution

Roya Nikfar designed the study and wrote the paper. Ahmad Shamsizadeh supervised the study and revised the paper critically. Manoochehr Makvandi, and Arash Khoshghalb collected specimens and performed the experiment.

Financial Disclosure

All authors have no conflicts of interest to declare, and do not have any financial or non-financial conflicts of interest.

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