Human Respiratory Syncytial Virus Infection and its Subgroups Among the Hospitalized Young Children With Acute Respiratory Infection

Seyed Mohammad Alavi 1,*, Manoochehr Makvandi 2, Saeid Najafi Fard 2, Leila Alavi 3

1Infectious and Tropical Diseases Research Center, Jundishapur University of Medical Sciences, Ahvaz, IR Iran
2College of Medicine, Jundishapur University of Medical Sciences, Ahvaz, IR Iran
3Food and Drug Deputy of Jundishapur University of Medical Sciences, Ahvaz, IR Iran
*Corresponding author: Seyed Mohammad Alavi, Infectious and Tropical Diseases Research Center, Jundishapur University of Medical Sciences; NO 52, 11 West Street, Ian Abad, Ahvaz, IR Iran. Tel: +98-6113387724, Fax: 06113335396, E-mail: alavi.seyedmohammad@yahoo.com.

Received: May 21, 2012; Revised: October 30, 2012; Accepted: December 15, 2012

Background: Respiratory syncytial virus (RSV) can cause acute respiratory infection (ARI) in infants and young children.

Objectives: This study was conducted to determine the incidence of RSV infection and its subgroups among children with ARI.

Materials and Methods: A total of 100 throat samples were collected from hospitalized children with ARI in different hospitals across the Khuzestan province from June 2009 to April 2010. The samples were tested for RSV by the nested PCR. The product of positive RSV was sequenced to determine the RSV subgroup, followed by phylogenic tree.

Results: Of total 100 patients, 29 (29%) including 16 (16%) male and 13 (13%) female were found positive for RSV infection. All the RSV positive patients were subgroup A dominant. High prevalence of RSV (8%) was found among the children under one year in contrast to 2% RSV incidence among the age group 6 years.

Conclusions: This study revealed that RSV subgroup A is dominant among the young children especially in children less than one year of age.

Keywords: Respiratory Syncytial Virus Respiratory Tract Infection; Children

Implication for health policy/practice/research/medical education: The results of this study are useful for health policy in acute respiratory infection control and management in young children.
3. Material and Methods

A total of 100 throat samples 54 (54%) male and 46 (46%) female were collected from the hospitalized children with ARI in the Khuzestan province from June 2009 to April 2010. Diagnosis of ARI was based upon National ARI guidelines and by pediatrics or trained general physician. The samples were tested for RSV by the nested PCR. The product of positive RSV was sequenced to determine the RSV subgroups and phylogenetic tree.

3.1. RNA Extraction

Viral nucleic acid was extracted from 200 μL of each sample using the High Pure Viral Nucleic Acid Kit (Roche Applied Science, Germany) according to the instructions. Then the cDNA was prepared.

3.2. The Nested PCR

The following G region conserved primers were used for the nested PCR (19).

- 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

For the first round, 25 μL of PCR master mix containing 1 μL of the cDNA of the each sample, 12.5 μL of the 1X PCR master mix (Sinagene company, Iran), primer G1 and G2 each 0.25 μL, D/W 11 μL. The PCR was performed for 40 cycles in Thermo cycler (Techne, UK), consisting initially 5 minutes for 95°C followed by, 1 minute at 52°C, 2 minutes at 72°C, 1 minute at 95°C and finally 5 minutes at 72°C for one cycle. The primers G3 and G4 were used for the second round. The 25 all of PCR master mix containing 1 μL of the PCR product of the first round, 12.5 μL of the 1X PCR master mixes, 0.25 μL of each G3 and G4 primers and D/W 11 μL.

The PCR was performed for 35 cycles consisting initially 5 minutes for 95°C followed by, 1 minute at 60°C, 1 minute at 72°C, 1 minute at 95°C and finally 5 minutes at 72°C for one cycle. The final product of 326 base pair (bp) showed positive test for PCR (16). The PCR product of the positive samples was sequenced to determine the RSV subgroup A or B. The phylogenetic and molecular evolutionary and nucleotide differences within and between the isolated sequences was carried out by using computer software of the Molecular Evolution Genetic Analysis (MEGA, Version 4) (17).

### Table 1. Distribution of RSV Positive Patients Among the Different Age Groups

| Age, y | <1, n = 20, No. (%) | 1 - 2, n = 25, No. (%) | 2 - 3, n = 16, No. (%) | 3 - 4, n = 11, No. (%) | 4 - 5, n = 10, No. (%) | 5 - 6, n = 18, No. (%) | Total, n = 100, No. (%) |
|--------|---------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Male   | 5/13 (5)            | 4/14 (4)               | 2/9 (2)                | 3/6 (5)                | 1/6 (1)                | 1/10 (1)               | 16/58 (16)             |
| Female | 3/7 (3)             | 3/11 (3)               | 3/7 (3)                | 2/6 (2)                | 1/4 (1)                | 1/8 (1)                | 13/42 (13)             |
| Total  | 8/20 (8)            | 7/25 (7)               | 5/16 (4)               | 5/11 (5)               | 2/10 (2)               | 2/18 (2)               | 29/100 (29)            |

3.3. Statistical Analysis

Statistical analysis was performed on PCR results and sequencing, data using either ANOVA followed by Bonferoni’s test for multiple comparisons or Student’s t-test using SPSS version 15, P value < 0.05 was indicated significant.

4. Results

Twenty nine (29%) patients including 16 (16%) male and 13 (13%) were showed positive results for RSV infection. The results of sequencing showed only subgroup A RSV was dominant among the patients. 8% prevalence of subgroup A was found among the children below one year compared to 2% RSV subgroup A prevalence in 6 years age group. No RSV subgroup B was found among the tested children. Distribution of RSV infection among the different age groups is shown in Table 1. The prevalence of RSV among the male and female was not shown significantly different (P > 0.05). The positive cases of RSV infection by nested PCR shown in Figure 1. The phylogenetic relationships of isolated RSV subgroup A from the children are shown in Figure 2.

![Figure 1. Detection of RSV Genome by Nested PCR With Specific Primer for G Region](image-url)
Figure 2. Phylogenetic Relationship of RSV A Viruses Isolated From the Children

The results of hyper variable region of G Amplicon PCR was sequenced (Bioneer, South Korea) and they found more similarity. We used nucleotide blast (www.ncbi.nlm.nih.gov) for genetic analysis. The phylogenetic analyses were performed by using MEGA version 5 (www.megasoftware.net) and phylogenetic tree was analyzed by the neighbor-joining method. Bootstrap reconstruction was done 1000 times to confirm the reliability of the phylogenetic tree.

5. Discussion

The present study showed that approximately a third of ARI cases in the region of study were infected with RSV. This means that RSV may be an important causative pathogen for ARI in this region of Iran. Our finding is in consistent with other studies reported RSV as an important pathogen responsible for ARI in young children (8-11).

The higher prevalence of RSV infection was found among the children below one year in contrast to RSV infection among the 6 years old children (8% vs. 2%, P < 0.05). Indeed, the risk of RSV infection in children below one year is greater than 6 years old children. This finding is in agreement with the previous studies reporting the higher RSV prevalence rate among the infants and children below 1 year old (12). Leader and et al. have reported the RSV as the leading cause of bronchiolitis among hospitalized infant (8).

Other studies revealed that the RSV is the most prevalent infection among the infants and children below 1 year old (8, 11, 12, 18). The relative frequency of RSV subgroup A and B is variable in annual outbreaks in all parts of the world. Both subgroups may be circulating in the same outbreak, or one type may clearly be dominant (15, 16). The results of phylogenic tree showed only the presence of subgroup A RSV which is in agreement with other studies (14-16, 18-20).

The detection of viral infections in ARI cases is increasing with advancement of sensitive screening of respiratory samples. As the sensitivity of diagnostic methods increase, the number of detected RSV associated ARI will continue to rise. Further investigation about the effect of RSV on respiratory infection will enable us to control this life threatening infantile infection. In the present study the role of other acute viral respiratory infection including Influenza A and B viruses, Adeno viruses, Para-influenza viruses, Rhino viruses, Corona viruses, Human metapneumo virus in the infants and children have not been studied however further investigations are recommended (21-25).

In conclusion, the RSV subgroup A was found to be dominant in this study however the additional large scale
studies are needed for further investigations.

Acknowledgments
We wish to thank Dr. Mokhtari, professor of faculty of the health, Tehran Medical University of Sciences, Iran, for kind cooperation in providing RSV (prototype RSV group A and B strains). We also thank all the pediatrics of Khuzestan province for diagnosing the children suspected to acute respiratory infection, and the chief and personnel of Infectious and Tropical Diseases research Center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran for approval and financially support of the study (grant No: 8908).

Authors’ Contribution
S. M. Alavi: design 80%, writing 60%, Analysis 70%; M. Makvandi: writing 30%, design 10%, lab working 50%; S. Najafi: lab work 50%; L. Alavi: design 10%, analysis 30%, writing 10%.

Financial Disclosure
We have no financial interests related to the material in this manuscript.

Funding/Support
This study was financially supported by Jundishapur Infectious and Tropical Diseases Research Center.

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