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Research Article

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

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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

1. Background

In the presence of risk factors such as infancy, young childhood and elderly respiratory syncytial virus (RSV) migrates from the upper respiratory track to the lower one, where the pathogenic effects of the virus, including airway inflammation that resulting airway occlusion, are amplified and can cause life-threatening bronchiolitis or pneumonia (1). RSV is probably the most significant respiratory pathogen of infants in the first 6 months of life and is responsible for a majority of hospitalizations and deaths in this age group in the world (2).

RSV causes a large burden of illness both in the community and in hospitals (3). As a routine, physicians approach acute respiratory infection (ARI) assuming a single-agent etiology, according to recent studies a substantial number of patients with ARI have more than one viral pathogen (4). The rate of viral co-infections varies between 10 - 60% (5, 6). Since clinical features of ARI with RSV are very similar to other different viruses, that make the clinical diagnosis complicated, and due to high rate of co-infection, RSV should be considered as a significant pathogen in differential diagnosis of child with ARI.

Respiratory syncytial virus (RSV) is a member of the family *Paramyxoviridae* (7). It infects epithelium airway and is responsible for ARI in infants, young children, asthmatics, and adults (8-10). RSV is the most common etiology in bronchiolitis (11). The acute infection of RSV induced bronchiolitis which may be leaded to hospitalization in 1 - 2% of infants and children (12). RSV can be divided into two major antigenic groups, A and B, based on nucleotide sequence differences (11, 13). This diversity is mainly found in the G surface glycoprotein (14). The subgroup A revealed to be the most prevalent worldwide (15). Knowing the influence of RSV on ARI helps the physicians to decide the best antiviral agent for management the patients and prevent unnecessary antibiotic consumptions against respiratory infections.

2. Objectives

Because the limited data about the viruses associated with ARI in Khuzestan province in south west of Iran, this study was conducted to determine the RSV infection and its groups among the hospitalized young children with ARI.

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 phylogenic 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

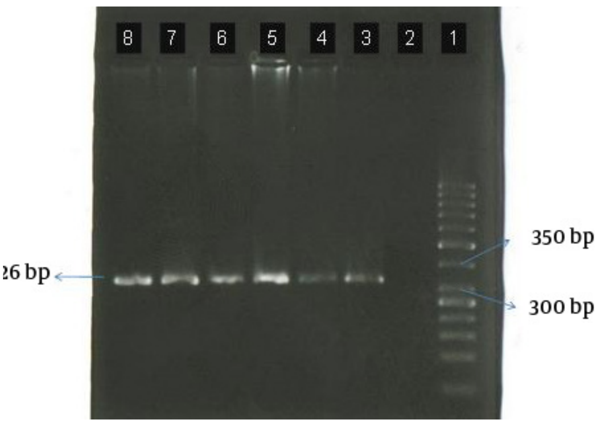
For the first round, 25 μL of PCR master mix containing 1 μL of the cDNA of the each sample, 12.5 μL of the 1X PCR master mix (Sinagene company, Iran), primer G1 and G2 each 0.25 μL, D/W 11 μL. The PCR was performed for 40 cycles in Thermo cycler (Techne, UK), consisting initially 5 minutes for 95°C 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 phylogenic 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 (3)	1/6 (1)	1/10 (1)	16/58 (16)
Female	3/7 (3)	3/11 (3)	3/7 (3)	2/5 (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)

Figure 1. Detection of RSV Genome by Nested PCR With Specific Primer for G Region



PCR products of 326 bp were detected in 2% agarose gel stained with ethidium bromide. Lane 1, DNA ladder; lane 2, negative control; lane 3, positive control; Lanes 4 to 6 were positive for RSV infection.

3.3. Statistical Analysis

Statistical analysis was performed on PCR results and sequencing, data using either ANOVA followed by Bonferroni'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.

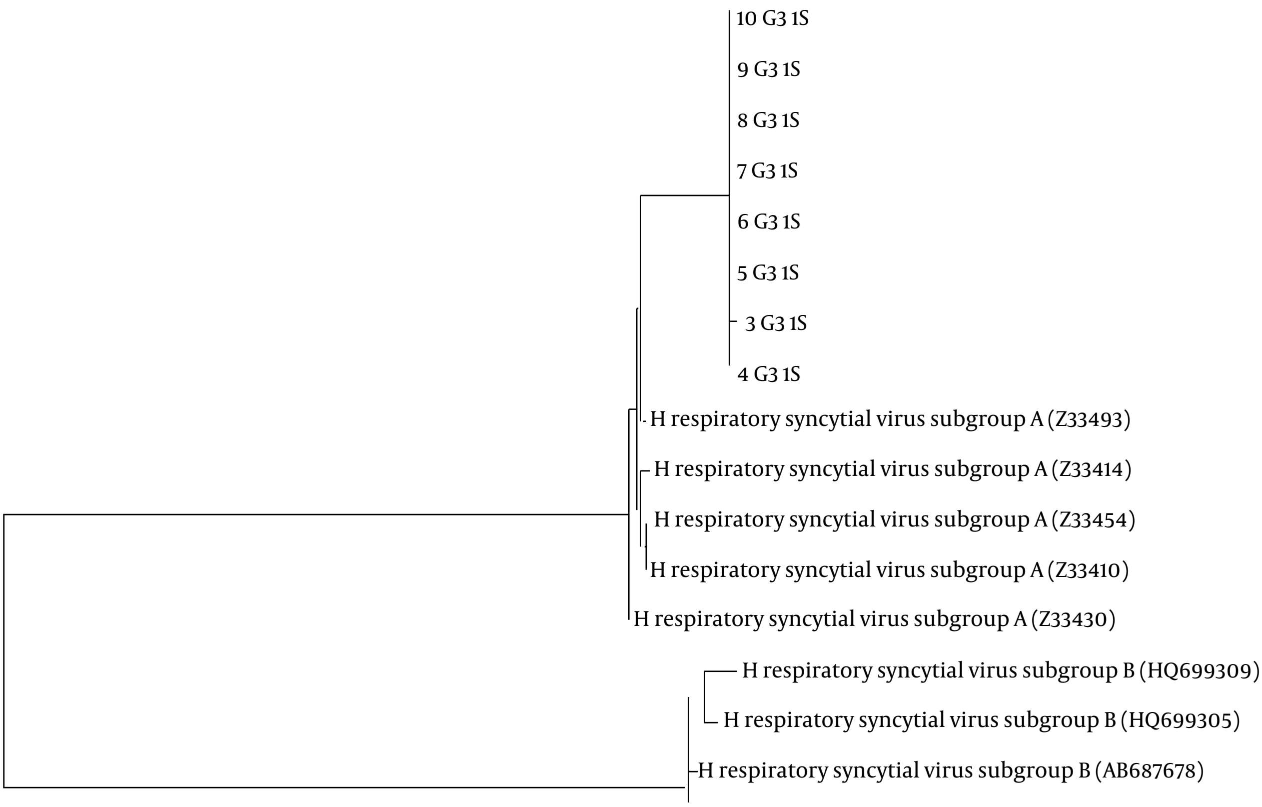


Figure 2. Phylogenetic Relationship of RSV A Viruses Isolated From the Children

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, Parainfluenza 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.

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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%.

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