Respiratory syncytial virus epidemiology in Turkey

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Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infections in infants and young children worldwide. This study was conducted to determine the prevalence of RSV among high-risk children admitted with respiratory symptoms in a developing country.

This is a multicenter study conducted among children less than 24 months of age and admitted to the hospital with respiratory symptoms. The inclusion criteria included: lower respiratory tract symptoms on admission, gestational age less than 35 weeks, and admission age less than six months, or children less than 24 months of age with a diagnosis of bronchopulmonary dysplasia requiring medical treatment or intervention during the last six months or with an uncorrected congenital heart disease (other than patent ductus arteriosus). Nasopharyngeal samples were obtained with one of the three standard methods: nasopharyngeal aspirate, nasopharyngeal wash or nasopharyngeal swab. RSV antigen was determined by enzyme immunoassay using Abbott TESTPACK RSV (No. 8100/2027-16). Statistical analysis was performed using Student's t-test and chi-square test.

In this study, 332 children (135 females, 40.7%; 197 males, 59.3%) were included, and the nasopharyngeal specimens of 98 (29.5%) children were determined to be RSV-positive. There were no differences in sex, age of gestation, age of admission, family education, number of siblings and smoking at home for RSV-positive and -negative cases. Furthermore, underlying disease and duration of hospital and intensive care unit stay were similar among groups. Only otitis media was more common among RSV-positive cases. No fatality at hospital was recorded. Frozen samples revealed more negative results. Most cases presented during winter and the number of RSV-positive cases was higher in cold and economically poor areas.

Premature children and children with underlying medical condition acquire RSV irrespective of other sociodemographic risk factors, and most of them are hospitalized. Thus, an RSV vaccine seems the most effective mode of protection to decrease morbidity and mortality.

Key words: respiratory syncytial virus, epidemiology.

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infections in infants and young children worldwide. The World Health Organization (WHO) estimates that of 12.2 million annual deaths in children under five years, a third are due to acute lower respiratory tract infections[1]. *Streptococcus pneumoniae, Haemophilus influenzae* and RSV are the predominant pathogens.

Respiratory syncytial virus is a seasonal virus, with peak rates of infection occurring annually in the cold season in temperate climates and in the hottest months and the rainy season in tropical countries. By two years of age it affects 90% of children; peak rates of infection occur in infants aged six weeks to six months and particularly in those under three months of age[2].

Respiratory syncytial virus has been etiologically linked with five clinical syndromes: mild upper respiratory tract illness, croup, bronchitis, bronchiolitis and pneumonia. As many as 40% of infants infected in the first six months of life will present with lower respiratory tract involvement, with bronchiolitis and pnemonia the most important manifestations. Repeated infections are common in all age groups, and previous infection does not prevent subsequent infections. Primary infection with RSV is usually more severe than reinfection[3].

In developed countries, high-risk groups in whom infection with RSV is more likely to progress into severe lower respiratory tract infections include infants with a history of premature birth; those with bronchopulmonary dysplasia, congenital heart disease or cystic fibrosis; immunosuppressed patients; individuals living in institutions; the elderly; and healthy infants under six months of age. Factors that increase frequency of infection are low socioeconomic status, crowded living conditions, indoor smoke pollution, and a family history of asthma or atopy[4,5].

Respiratory cyntial virus has also been shown to be the most frequent cause of lower respiratory tract infections in children under five in developing countries. However, in developing countries the risk factors are not well defined and need further studies[6].

This study was conducted to determine the prevalence of RSV among high-risk children admitted with respiratory symptoms in a developing country.

Material and Methods

This is a multicenter study conducted between 2000 and 2002 in 17 centers among children less than 24 months of age admitted to the hospital with respiratory symptoms. The inclusion criteria included: lower respiratory tract symptoms on admission, gestational age less than 35 weeks, admission age less than six months, or children less than 24 months of age with a diagnosis of bronchopulmonary dysplasia requiring medical treatment or intervention during the last six months or with an uncorrected congenital heart disease (other than patent ductus arteriosus). Exclusion criteria included children with a known bleeding dyscrasia, those treated with RSV-intravenous immunoglobulin (IVIG) or palivizumab during the last six months or still under treatment, those whose nasopharyngeal sampling was not performed within 24 hours of admission, and those without respiratory symptoms. The calculated sample size was 385, but 400 children were planned to be included. The sample size was calculated by estimating the lower respiratory tract infection due to RSV to be 50% with a p value of 0.05.

Parents were informed of the study and informed consent was obtained before inclusion of all cases. After inclusion all cases were recorded to case report form (CRF).

Nasopharyngeal samples were obtained with one of the three standard methods: nasopharyngeal aspirate, nasopharyngeal wash or nasopharyngeal swab[7]. All samples were sent to the laboratory in viral transport media including Hank's balanced salt solution (pH 7.2), on wet ice. Samples were either stored at 4°C and processed within 24 hours or stored at –20°C for later processing.

Respiratory syncytial virus antigen was determined by enzyme immunoassay using Abbott TESTPACK RSV (No. 8100/2027-16)[8].

Statistical analysis was performed by Student's t-test and chi-square test.

Results

In this multicenter prospective study, 332 children (135 females, 40.7%; 197 males, 59.3%) were included. Mean age of the children was 5.9±5.1 months (range 0-36 mos). The mean gestational age of these children was 36.0±4.1 weeks (range 24-42 weeks). None of the children had been administered RSV prophylaxis earlier. Among these 332 children, 36 (11%) had bronchopulmonary dysplasia (BPD), 69 (20.8%) congestive heart failure, 3 (0.95%) cystic fibrosis, and 10 (3%) immune deficiency. The remaining 214 (64.3%) were included in the study as they were born before 35 weeks of gestational age.

Among these 332 children, the nasopharyngeal specimens of 98 (29.5%) children were revealed to be RSV-positive.

Females were more prone to RSV than males (36.3% vs 25%, p=0.027). Although children positive for RSV tended to have a lower mean gestational week at birth, the difference was not significant (35.5±4.2 vs 36.3±4.0 weeks, p>0.05). Similarly, regarding the age

of patients on admission, although positive cases tended to be younger, the difference was not significant (5.2±5.1 vs 6.2±5.1 mos, respectively, p>0.05).

No difference between positive and negative patients could be demonstrated for family education, number of siblings and smoking at home (Table I).

Table I. RSV Positivity and Some Sociodemographic Characteristics

Characteristic	RSV Negative n (%)	RSV Positive n (%)
Family education		
Primary school	110 (55.3)	47 (52.2)
High school	72 (36.2)	35 (38.9)
University	17 (8.5)	8 (8.9)
Number of siblings		
≤1	139 (60.4)	66 (67.3)
2-3	63 (27.4)	22 (22.4)
≥ 4	28 (12.2)	10 (10.2)
Smoking at home		
No	106 (45.9)	50 (51.0)
Yes	125 (54.1)	48 (49.0)

p>0.05.

The clinical presentations of RSV-positive and -negative cases are compared in Figure 1. Only presence of otitis media was more common among RSV-positive cases.

Underlying disease did not increase the risk of RSV positivity in our study population (Table II).

There was no difference between RSV-positive and -negative cases regarding duration of intensive care unit stay (Table III), duration of respiratory support (Table IV) and total duration of hospital stay (12.05±15.58 vs 11.15±10.05, respectively, p>0.05). There were no fatalities among the cases during hospital stay.

Table II. Underlying Medical Condition According to RSV Positivity

Disease	RSV Negative n (%)	RSV Positive n (%)
BPD	29 (32.6)	7 (24.1)
CHF	50 (56.2)	19 (65.5)
Cystic fibrosis	2 (2.3)	1 (3.5)
Immune deficiency	8 (8.9)	2 (6.9)

p>0.05.
BPD: Bronchopulmonary dysplasia.
CHF: Congestive heart failure.

Table III. Duration of Intensive Care Unit Stay According to RSV Positivity

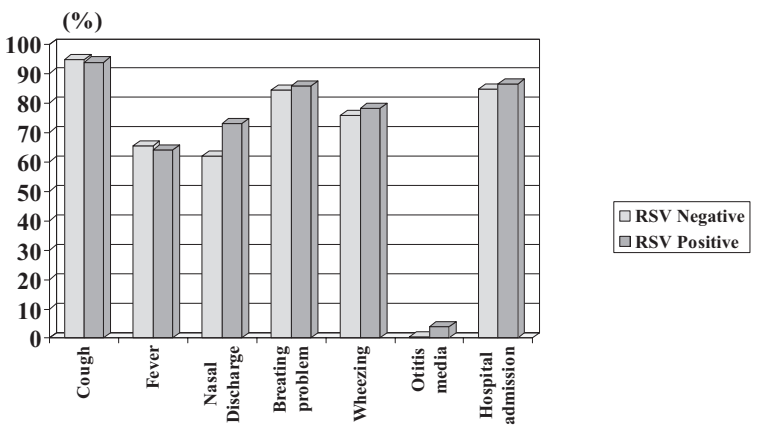
Duration (days)	RSV Negative n (%)	RSV Positive n (%)
0	128 (59.8)	54 (60.7)
1	20 (9.3)	9 (10.1)
2-4	31 (14.5)	9 (10.1)
5-7	9 (4.2)	7 (7.9)
>8	26 (12.2)	10 (11.2)

p>0.05.

Table IV. Duration of Respiratory Support According to RSV Positivity

Duration (days)	RSV Negative n (%)	RSV Positive n (%)
0	179 (82.3)	83 (92.2)
1	16 (7.4)	2 (2.2)
2-4	15 (7)	4 (4.4)
5-7	1 (0.5)	0 (0)
>11	4 (1.8)	1 (1.1)

p>0.05.



• Only otitis media was significantly more common among RSV-positive cases, p=0.029.

Fig. 1. Clinical caharacteristics of children on admission according to RSV positivity.

Although the mode of sampling did not affect positive results, frozen samples were more often negative (Table V). Time between sampling and analysis was longer for RSV-negative cases (8.9±22.5 vs 0.042±0.2 days, respectively, p<0.0001).

Although the year of admission did not differ for RSV-positive cases, there was a statistically significant difference regarding the months of admission (Fig. 2). Positivity was higher for the first three months and the last month of the year, representing the winter season.

There was also a difference in the RSV-positive cases by region. In centers from which more than 20 cases were included in the study, RSV positivity was higher among the colder and poor socioeconomic regions than in temperate zones (p<0.01) (Table VI).

Table VI. Regional Distribution of RSV-positive Cases (Centers from which more than 20 cases were included)

Hospital (Province)	RSV Negative n (%)	RSV Positive n (%)
Dicle (Diyarbakır)	47 (88.7)	6 (11.3)
Erciyes (Kayseri)	16 (76.2)	5 (23.8)
Hacettepe (Ankara)	39 (60.9)	25 (39.1)
100.Yıl (Van)	29 (54.7)	24 (45.3)
19 Mayıs (Samsun)	23 (95.8)	1 (4.2)

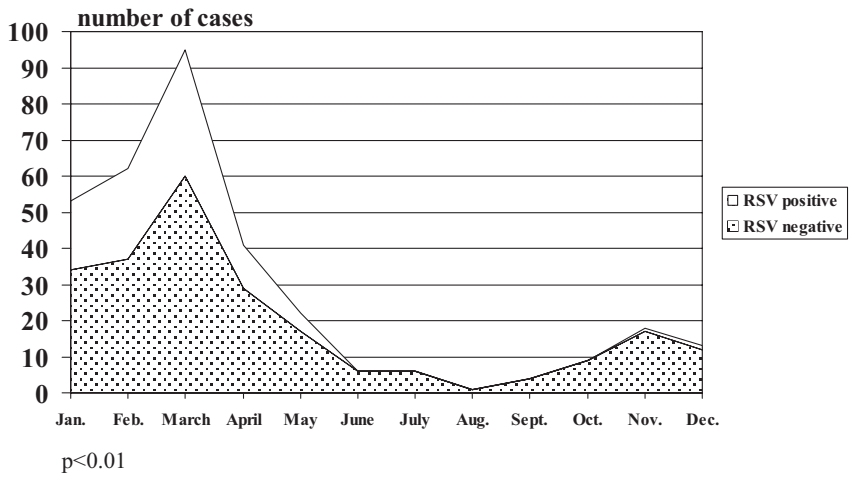
p<0.001.

studies from Turkey as it is a multicenter study including cases from all over the country. It is thus representative for Turkey.

No difference could be found in gestational age and age on admission. It has been reported that although in developed countries young infants are a risk group for RSV, in developing countries children under five years of age are at risk[4,6]. Since some regions in Turkey are developed and others developing, and since our study was representative of the country, no age difference could be found for RSV-positive cases as the target population was under 24 months of age. Furthermore, no difference for positive and

Table V. RSV Positivity According to Sampling

Sampling	RSV Negative n (%)	RSV Positive n (%)	
Sampling type			
Nasal aspiration	109 (47.0)	50 (51.0)	>0.05
Nasal washing	79 (34.0)	37 (37.8)	
Nasal swab	44 (19.0)	11 (11.2)	
Sample storage			
Fresh	204 (88.3)	94 (96.9)	0.018
Refrigeration <24 hr	4 (1.7)	2 (2.0)	
Frozen	23 (10)	1 (1.1)	



p<0.01

Fig. 2. RSV positivity according to months.

Discussion

In this prospective multicenter study, the RSV positivity among high-risk patients admitting with respiratory tract symptoms was found to be 29.5%. In fact, this finding is comparable with other studies from either Turkey or foreign countries[4,6,9,10]. This study differs from other

negative cases could be found regarding family education, number of siblings and smoking at home. This can be attributed to the characteristics of our study population, in which the cases were already high-risk patients due to underlying disease or gestational age. So we can speculate that these host factors may be significantly more important than other demographic characteristics regarding disease acquisition.

In this study, samples for RSV testing were obtained by three methods - nasal aspiration, nasal washing and nasal swab. No difference in RSV positivity could be determined according to mode of sampling. Although other studies have reported a lower rate of positivity for swab specimens[7], we could not find a statistically significant difference. This may also be due to the limited number of samples obtained by swab compared to other studies.

Enzyme immunoassay Abbott Test-pack was used for studying RSV antigen. The sensitivity and specificity has been demonstrated to be high using this method[7,8]. Samples were stored in three ways - fresh, refrigeration for less than 24 hours, and frozen. It was shown that the rate of positivity was lowest among frozen samples. There was no difference in positivity among fresh specimens and those refrigerated for less than 24 hours. This finding is also compatible with earlier studies. Thus, although Test-pack is a simple and reliable method for clinical studies, samples should be taken within 24 hours of admission. It is recommended that samples not be frozen but rather studied within 24 hours of sampling.

While frequency of cough, fever, nasal discharge, breathing problems and wheezing was similar in both groups, otitis media was much more common among RSV-positive cases. This finding is supported by other studies in which otitis media was a common manifestation of RSV virus in young children[11]. On the other hand, there was no difference in hospital admission rate, duration of hospital and intensive care unit stay, or duration of mechanical ventilation among RSV-positive and -negative cases. In other studies, the hospital duration differed, but this can be explained by the different criteria used for hospitalization between different countries and institutions[4,11]. Thus, it is difficult to compare our results with other countries. On the other hand, if we discuss our findings within our country, finding no difference between RSV-positive and -negative cases may again be related to our study population characteristics. As outlined earlier, all of our study population were high-risk patients (prematurity or underlying disease). Therefore, not only RSV but many other respiratory pathogens outside the scope of this study may have necessitated hospitalization.

The distribution of our cases was comparable to that observed in other temperate climates, with an increase in cases in the cold season. The number of cases peaked between January to April, and there were no cases between June and October. In addition, there was also a regional predilection for colder regions of the country.

In conclusion, the number of RSV cases is increased during the cold season. Compared to other respiratory tract symptoms, otitis media is much more common in cases with RSV positivity. Test-pack is a simple and reliable method of diagnosis when samples are taken early and studied within 24 hours of sampling. Premature children and children with underlying medical condition acquire RSV irrespective of other sociodemographic risk factors and most of them are hospitalized. Thus, an RSV vaccine seems the most effective mode of protection to decrease morbidity and mortality.

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