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Investigation of respiratory syncytial virus in children with respiratory tract infection by real-time polymerase chain reaction

Solunum yolu enfeksiyonu olan çocuklarda respiratuvar sinsityal virusun gerçek zamanlı polimeraz zincir reaksiyonu ile araştırılması

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Abstract

Aim: Respiratory syncytial virus (RSV) is the most common agent of respiratory tract infections (RTIs) in the early stages of life. This study aimed to investigate RSV A/B in children with RTIs by multiplex Polymerase Chain Reaction (PCR) and evaluate the distribution of RSV among age groups, concurrent co-infections, and features of its seasonal distribution.

Methods: Zero to eighteen-year-old patients whose nasopharyngeal swab samples were analyzed with the pre-diagnosis of RTI between April 2015 - March 2018 were included in this cross-sectional study. RSV A/B and other viruses of the respiratory panel were investigated with the multiplex real-time PCR method.

Results: Median age of 2707 patients was 1 (age range: 0-18) and 57.4% (1554) of them were male. RSV positivity was found in 14.4% (390). Prevalence of RSV in females and males were 13.6% and 15.5% respectively (P=0.16). The highest RSV rate was 18.1% in those younger than one year. Mixed infection factors were found in 5.4%, the most common ones being RSV and human rhinovirus (1.8%). RSV was mostly seen in December, followed by January and February (P<0.001).

Conclusion: RSV was found in 14.4% of the pediatric patients with RTI. We found that RSV was more common under the age of 1. In this study, we determined that RSV was commonly seen in winter. Early diagnosis of RSV with real-time PCR plays a crucial role in the prevention of unnecessary use of antibiotics and nosocomial infections. It may also help to implement an effective approach to the prevention, control, and treatment of RTIs during winter when the incidence increases.

Keywords: Respiratory syncytial virus, Real-time PCR, Respiratory tract infection

Öz

Amaç: Respiratuvar sinsityal virus (RSV), yaşamın ilk yıllarında solunum yolu enfeksiyonlarının (SYE) en sık etkenidir. Bu çalışmada SYE olan çocuklarda RSV A/B'nin multipleks polimeraz zincir reaksiyonu (PCR) ile araştırılması, yaş grupları arasında RSV dağılımı, eşlik eden koenfeksiyonlar ve mevsimsel dağılım özelliklerini değerlendirmeyi amaçladık.

Yöntemler: Bu çalışma çapraz kesitsel olarak planlanmıştır. Çalışmaya Nisan 2015 – Mart 2018 tarihleri arasında, 0-18 yaş arasında SYE ön tanısı ile gönderilen nazofaringeal sürüntü örnekleri dahil edildi. Örneklerden nükleik asit ekstraksiyon işlemi, EZ1 Virus Mini Kit V 2.0 (Qiagen, Almanya) ile EZ1 Advanced XL (Qiagen, Almanya) cihazında yapıldı. RSV A/B ve solunum paneline ait diğer viruslar, FTD Respiratory pathogens 21 (Fast-Track Diagnostics, Luxembourg) kiti kullanılarak multipleks real-time PCR yöntemi ile araştırıldı.

Bulgular: Yaş aralığı 0-18 olan 2707 hastanın medyan yaşı 1 idi ve %57,4'ü (1554) erkekti. RSV pozitifliği %14,4'ünde (390) tespit edildi. Kız ve erkeklerde RSV sıklığı sırasıyla %13,6 ve %15,5 idi (P=0,16). En yüksek RSV oranı <1 yaş grubunda %18,1 idi (P<0,001). Mikst etken ile enfeksiyonu %5,4'ünde saptandı. En fazla RSV ve HRV (%1,8) birlikteliği görüldü. RSV en sık Aralık (%27,9), ardından Ocak ve Şubat ayında saptandı (P<0,001).

Sonuç: SYE olan pediatrik hastaların %14,4'ünde RSV saptadık. RSV'nin 1 yaş altında daha yaygın olduğu tespit ettik. Bu çalışmada, RSV'nin kış mevsiminde sık görülen bir patojen olduğunu belirledik. Real-time PCR ile RSV tanısının erken konulması, gereksiz antibiyotik kullanımının ve nozokomiyal enfeksiyonların önlenmesi açısından önem taşımaktadır. Ayrıca, insidansın arttığı kış mevsimi boyunca SYE'lerin önlenmesi, kontrolü ve tedavisi için etkili bir yaklaşımın uygulanmasına yardımcı olabilecektir.

Anahtar kelimeler: Respiratuvar sinsityal virus, Real-time PCR, Solunum yolu enfeksiyonu

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Introduction

Respiratory syncytial virus (RSV) is an enveloped, negative-stranded RNA virus of the *Pneumoviridae* family [1]. RSV is known as an important respiratory tract pathogen since it was isolated from infected children in 1957 [2,3], which affects all age groups. Virus transmission occurs through contact with infected persons or contaminated materials through the eye, nose or mouth mucosa. Inhaled fomites may be the cause of infection, which is generally limited to the upper respiratory tract [4]. However, it may also evolve into a serious lower respiratory tract infection (RTI). RSV constitutes the most common cause of pneumonia and bronchiolitis in children [5]. Sixty-seventy percent of the children under the age of 1 and almost all children under the age of 2 are affected by this virus [6]. RSV is associated with recurrent wheezing and pediatric asthma [7].

According to the data of 2015, it is estimated that RSV is responsible for 33.1 million lower RTI attacks, 3.2 million admissions to hospital and 118.200 deaths under the age of 5 around the world [8]. Although it is reported that the rate of RSV infection is higher in pediatric patients, some life-threatening infections may be seen in healthy children and the elderly, especially over the age of 65 [9]. Comorbid conditions such as congenital heart disease, chronic lung disease, Down syndrome, premature birth, cystic fibrosis and immune deficiency may increase morbidity and mortality [8,10-12]. RSV infection may cause more serious symptoms in cases of bacterial infection and superinfection [13]. Factors such as low education, decreased family income, and residing in crowded environments increase the probability of the transmission of RSV infection. It is concluded that there is a strong relationship between RSV infection and seasonal changes [14]. The prevalence of the infection differs according to geographical location, climate changes, genetic disposition, socioeconomic factors and regional virus strains [15].

In this study, a retrospective epidemiological analysis was performed to determine the prevalence of RSV in the age group of 0-18, outpatients and inpatients, its seasonal distribution and its peak season as well as the frequency of concurrent co-infections.

Materials and methods

Zero to eighteen-year-old patients whose nasopharyngeal swab samples were analyzed with the pre-diagnosis of RTI between April 2015 and March 2018 in the Medical Microbiology department were included in this cross-sectional study. RSV and other viruses of the respiratory panel such as Adenovirus, Bocavirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Enterovirus, Human metapneumovirus A/B, Influenza A, Influenza A (H1N1), Influenza B, Parainfluenza 1-4, Parechovirus, and RSV A/B were investigated with multiplex real-time PCR using FTD Respiratory pathogens 21 (Fast-Track Diagnostics, Luxembourg) kit in nasopharyngeal swab samples sent to the Virology Laboratory. Samples, which were transported under appropriate conditions for the microbiology laboratory, were stored at +4˚C until analysis. Viral genome extraction was performed by Qiagen EZ1 Virus Mini Kit v2.0 (Qiagen, Germany). The product obtained after the extraction underwent an amplification process in Rotor Gene Q Real-Time PCR device (Qiagen, Hilden, Germany).

This study was performed with the approval of the Non-Interventional Clinical Research Ethical Committee of Istanbul University (reference number: 2018/08/662).

Statistical analysis

SPSS 25 (SPSS Inc, Chicago, IL, USA) package program was used for statistical evaluation of the data. Continuous data were given as mean (standard deviation), and categorical data were given as number and percentage. Visual properties (histogram and probability graphs) and Kolmogorov-Smirnov test were used for normal distribution of variables. Student's T test or Mann-Whitney U test were used to compare the variables. Qualitative variables were compared using Pearson Chi-Square or Fisher exact tests. A *P*-value of <0.05 was considered statistically significant.

Results

A total of 2707 respiratory tract samples were obtained from 1863 (68.8%) outpatients and 844 (31.2%) inpatients. Median age of the pediatric patients in this study was 1 (age range: 0-18). One thousand, five hundred and fifty-two (57.3%) were males and 1155 (42.7%) were females. 390 out of 2707 pediatric patient samples (14.4%) were RSV positive. The prevalence of RSV in females and males were 13.6% (211) and 15.5% (179) respectively (P=0.16). Median age of the patients with RSV infection was 1. The highest rate of RSV was seen in patients under the age of 1, which was statistically significant (P<0.001).

Single infection with RSV was found in 9.1% (244/2707) of the patients and multiple factors with identified in 5.4% (146/2707). The rate of single and concurrent RSV infections were 8.8% (31/352) and 2.8% (10/352), respectively, in the age group of 6-10 years. The difference between the two was statistically significant (P=0.04) (Figure 1). Human rhinovirus (HRV) was the most commonly found pathogen concurrent with RSV (P<0.001) (Table 1). HRV was isolated in 59 of RSV-positive respiratory samples, human metapneumovirus was isolated in 16 and HBoV, in 15 samples. Parechovirus was not found in any RSV-positive patients (Table 2).

Among outpatients and inpatients, the rates of single infection with RSV were 8.7% (162/1863) and 9.8% (83/844) respectively, while the rates of concurrent infection were 5.8% (108/1863) and 4.4% (37/844) respectively (P=0.22) (Table 2). The most common multiple infection factor in RSV-positive respiratory samples was HRV (15.1%) (P=0.12) (Table 2, Figure 2).

Forty-one out of 120 inpatients had a comorbid disease, 10 of which were diagnosed with lower RTI (bronchiolitis and pneumonia). The most common comorbid diseases in patients with RSV infection were congenital metabolic diseases, renal diseases, hematologic malignancy and neurological disorders (Table 3).

When the distribution of RSV positivity according to the months was evaluated, it was found that RSV was mostly seen in December (27.9%), followed by January (21.3%) and

February (17.2%) (P<0.001). The lowest positivity rates were detected in July (0.8%) and August (1.4%) (Figure 3). Multiple infections with RSV were mostly seen in January (9.9%) and December (9.8%) (P<0.001).

Table 1: Demographic and laboratory features of the patients

		% (n)	*P*-value
RSV positive patients	Total	14.4 (390/2707)	
	Male	13.6 (211/1552)	0.16
Age (median)		1(range=0-18)	
Single infection		9.1 (244/2707)	0.29
Multiple infection		5.4 (146/2707)	
Double pathogens	RSV+HRV	1.8 (50)	<0.001
	RSV+EV	0.04 (1)	
	RSV+HBoV	0.3 (8)	
	RSV+HCoV-229E	0.2 (6)	
	RSV+HCoV-HKU1	0.2 (6)	
	RSV+HCoV-NL63	0.04 (1)	
	RSV+HCoV-OC43	0.1 (2)	
	RSV+hMPV	0.5 (14)	
	RSV+ADV	0.2 (5)	
	RSV+INF A	0.5 (13)	
	RSV+INF A H1N1	0.4 (11)	
	RSV+INF B	0.1 (3)	
	RSV+PIV-1	0.1 (2)	
	RSV+PIV-2	0.04 (1)	
	RSV+PIV-3	0.1 (2)	
	RSV+PIV-4	0.1 (2)	
Triple and four pathogens	RSV+ADV+EV	0.1 (2)	
	RSV+ADV+hMPV	0.04 (1)	
	RSV+ADV+INF-A H1N1	0.04 (1)	
	RSV+HBoV+hMPV	0.04 (1)	
	RSV+HBoV+INF-A H1N1	0.1 (2)	
	RSV+ADV+HCoV-229E	0.04 (1)	
	RSV+HCoV-NL63+HCoV-229E	0.04 (1)	
	RSV+HCoV–HKU1+PIV-4	0.04 (1)	
	RSV+HRV+HCoV–HKU1	0.1 (2)	
	RSV+HRV+ADV	0.04 (1)	
	RSV+HRV+EV	0.04 (1)	
	RSV+HRV+HboV	0.1 (4)	
	RSV+HRV+HCoV-229E	0.04 (1)	
	RSV+INF-A+HCoV–HKU1+PIV-4	0.04 (1)	

HRV: human rhinovirus; INF-A: influenza A virus; H1N1: pandemic influenza A H1N1 virus; hMPV: human metapneumovirus; HBoV: human bocavirus; HCoV: human coronavirus; PIV: parainfluenza virus ADV: adenovirus

Table 2: Distribution of multiple infections detected with Respiratory syncytial virus (RSV) positive samples in outpatients and inpatients

Infection	Outpatient n (%) 1863	Inpatient n (%) 844	*P*-value
Single infection	162 (8.7)	83 (9.8)	0.22
Multiple infection	108 (5.8)	37 (4.4)	
Human rhinovirus	40 (2.1)	19 (2.3)	0.80
Human metapneumovirus	11 (0.6)	5 (0.6)	1.00
Human Bocavirus	11 (0.6)	4 (0.5)	1.00
Influenza virus type A H1N1	11 (0.6)	3 (0.4)	0.56
Influenza virus type A	10 (0.5)	4 (0.5)	1.00
Adenovirus	9 (0.5)	2 (0.2)	0.52
Coronavirus-HKU1	8 (0.4)	2 (0.2)	0.73
Coronavirus-229E	7 (0.4)	1 (0.1)	0.44
Coronavirus-OC43	3 (0.2)	1 (0.1)	1.00
Parainfluenza virus type 4	2 (0.1)	2 (0.2)	0.59
Influenza virus type B	3 (0.2)	0 (0)	0.56
Coronavirus- NL63	1 (0.1)	1 (0.1)	0.52
Enterovirus	2 (0.1)	0 (0)	1.00
Parainfluenza virus type 1	2 (0.1)	0 (0)	1.00
Parainfluenza virus type 3	2 (0.1)	0 (0)	1.00
Parainfluenza virus type 2	1 (0.1)	0 (0)	1.00
Parechovirus	0 (0)	0 (0)	-

Table 3: Distribution of comorbid diseases in RSV-positive inpatients (n=43)

Comorbid diseases		n (%)
Congenital metabolic disease		9 (20.9)
	Propionic acidemia	4
	Maple syrup urine disease	1
	Fructose 1.6 diphosphatase	1
	Gaucher	1
	Metabolic disease, undefined	2
Renal disease		4 (9.3)
	Chronic renal failure	1
	Membranoproliferative glomerulonephritis	1
	Renal transplantation	2
Hematologic malignancy		4 (9.3)
	Acute lymphoblastic leukemia	4
Neurologic disease		4 (9.3)
	Cerebral palsy	1
	Epilepsy	1
	West syndrome	1
	Guillain-barre sydrome	1
Congenital heart disease		2 (4.7)
	Fallot tetralogy	1
	Ventricular septal defect	1
Liver disease		2 (4.7)
	Liver transplantation	2
Chronic lung disease		2 (4.7)
	Asthma	2
Other		16 (37.2)
	Inguinal hernia, umbilical hernia, cleft palate, pyloric stenosis, biliary atresia, low-birth-weight baby, premature baby	
Total		43

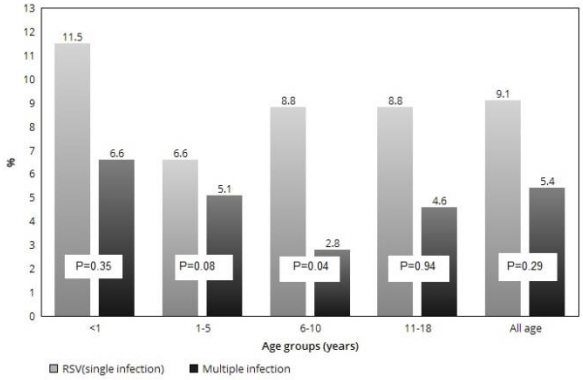

Figure 1: Distribution of single-infection (RSV) and multiple-infections according to the age groups

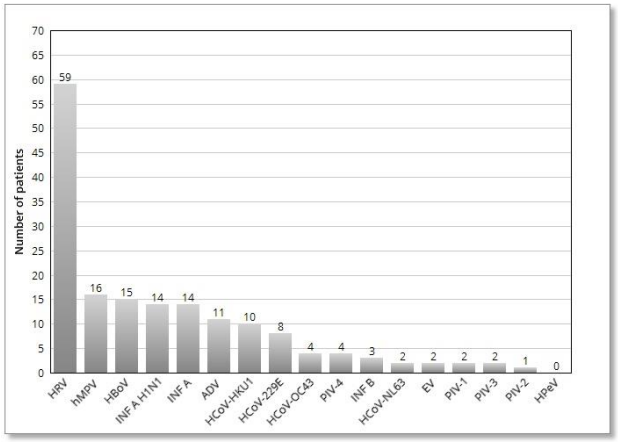

Figure 2: Distribution of co-infections in RSV-positive samples (number) (HRV: human rhinovirus, hMPV: human metapneumovirus, HBoV: human bocavirus, INF: influenza, ADV: adenovirus, HCoV: human coronavirus, PIV: parainfluenza virus, EV: enterovirus, HPeV: human parechovirus)

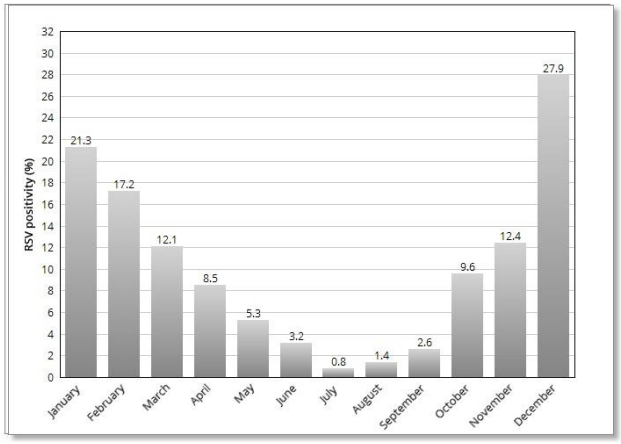

Figure 3: Distribution of human RSV according to the months

Discussion

RSV is the most common factor of RTIs in the early ages of life. Epidemiological studies are crucial as they help determine the timing of RSV outbreaks. In the studies conducted in our country, viral etiology is observed at a rate between 41.8% and 85% in acute RTIs. Also in these studies, RSV was found at a rate between 0% and 63% in different age groups (Table 4) [16-23]. In our study, we found RSV in 14% (390/2707) of the children diagnosed with RTI in the age group of 0-18. The highest rate of RSV was 18.1% (92/1060) in those younger than one year. Studies report that more than 80% of the children have RSV infection until the age of 2 [24]. Age and gender are important risk factors for serious RSV disease and children and the elderly are the groups at the highest risk for serious complications of the infection [25,26]. Incidence of RTI secondary to RSV decreases by age until late adulthood [27,28].

Asymptomatic infection is more commonly seen in adults and may contribute to the spread of the infection [29]. In our study, we did not find any difference between genders in terms of positivity rates of RSV infection.

Table 4: Characteristics of RSV infections in patients in Turkey

Location	Viral etiology %	Age	Respiratory tract infection	Prevalence (RSV)	Years	References
Aegean region						
Izmir	41.8	0-10 years	Lower	61	2009-2010	Akçalı S. et al. [22]
Izmir	75	<24 months	Lower	76.24	2013-2016	Gökçe Ş. et al. [23]
Central Anatolia						
Ankara†	25/39	20-67	Upper/ Lower	32	2013	Atilla E. et al. [19]
Ankara‡	51.8	90.4± 63.1 months	Upper/ Lower	17/86	2012-2014	Aldemir Kocabaş B. et al. [20]
Konya	58.2	0-18	Acute	ᵠ17.8, 27.9	2013-2015	Tüzüner U. et al. [21]
Marmara region						
Istanbul	85*	All age 0-5 6-23 24-59 ≥60	Upper	13.5 50 13 27 0	2005-2006	Ünüvar E. et al. [16]
Istanbul	78.6	2-16 years	Upper/ Lower	13–9.2	2013-2014	Aktürk H. et al. [17]
Sakarya		1-24 months	Lower	63	2018	Karakoyun M. et al. [18]
In this study (Istanbul)		0-18 years	Upper/Lower	14.4	2015-2018	

*Acute upper respiratory tract infections, †Adult recipients of allogeneic hematopoietic stem cell transplantation (Allo-HSCT) upper and lower respiratory tract infections, ‡The pediatric hematology and oncology department, ᵠ RSV-A, RSV-B respectively

The incidence of RSV differs according to the geographical regions and seasonal differences around the world. In mild climates, it is significantly more common in winter and spring. RSV outbreaks are very rare between June and September [30]. It causes outbreaks in the northern hemisphere between November and April and in southern hemisphere between March and October [31,32]. In the studies conducted in tropical regions, it is asserted that high humidity and year-round high temperatures allow air-borne transmission via droplet nuclei throughout the year [33]. In this study, we found that the highest and lowest rates of RSV positivity occurred between December-February and July-August, respectively. In a study in which the seasonal distribution of RTIs in Izmir in Aegean Region was investigated, the respiratory viruses were mostly isolated in winter (44.4%) and least during the summer (8.3%). Similarly, multiple infection factors were mostly seen in winter (46.9%) and least during the summer (8.8%) [34]. RSV was mostly found in Autumn (40%) and least during summer in children pre-diagnosed with RTI under the age of 5 in Konya, the Central Anatolia Region [10,35]. It was found that average RSV season began in early December and continued until early April in 15 European countries [36].

RSV infection is more severe in patients with a comorbid disease. Groups at the highest risk are children and the elderly with suppressed immune systems or cellular immunodeficiency. Low socioeconomic condition, malnutrition and some environmental factors such as crowded residential conditions and indoor air pollution may also lead to the development of more severe disease [37,38]. Prematurity, congenital heart diseases, congenital lung anomalies, cystic fibrosis, pulmonary malformations, and neurogenic disorders render children more prone to severe RSV disease [37]. In this study, the comorbid diseases in inpatients with RSV infection were mostly congenital metabolic diseases, renal diseases, hematologic malignancies, and neurological disorders.

Limitations

The retrospective nature of this study was the primary limitation, which made it impossible to distinguish the upper and lower RTIs due to the fact that no such records existed in the hospital registry.

Conclusion

RSV positivity was found in 14.4% of the samples of pediatric patients in the age group of 0-18 years. Consistent with the literature, we found that RSV was more common under the age of 1. We also found that RSV was a pathogen mostly seen in winter. Early diagnosis of RSV with real-time PCR is crucial in terms of the prevention of unnecessary use of antibiotics and nosocomial infections, as it allows timely intervention to control the spread of the disease. Further epidemiological studies are needed.

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