Acute lower respiratory tract infections caused by PCR proven RSV and non-RSV viruses in the NICU

Yenidoğan Yoğun Bakım Ünitesinde PCR ile Kanıtlanmış RSV ve RSV dışı Virüslerin Neden Olduğu Akut Alt Solunum Yolu Enfeksiyonları

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ABSTRACT

Objective: In recent years, detection of viruses by multiplex real time polymerase chain reaction allowed the isolation of causative viral agents.

The primary objectives of this study were to determine the distribution of causative respiratory viruses in acute lower respiratory tract infections in the NICU and to compare the demographic and clinical characteristics of infants with RSV and non-RSV infections. The secondary aim was to determine risk factors requiring respiratory support.

Material and Methods: This retrospective observational study was conducted between January 2016 and June 2019. The infants were divided into two groups as RSV and non-RSV, and compared. Risk factors for respiratory support by means of invasive/non-invasive ventilation were determined.

Results: Two hundred forty-three infants were hospitalized with the diagnosis of viral lower respiratory tract infections and a total of 119 infants, in which a causative viral agent could be isolated, were included in the study. RSV was the most common detected virus (n=93, 78%). The demographic characteristics of infants in RSV and non-RSV groups were similar, except postnatal age. Infants were hospitalized most frequently in between December and February. In multivariate analysis, apnea and RSV were found to be risk factors for respiratory support requirement.

Conclusion: Since RSV-associated acute lower respiratory tract infections are the most common and require more respiratory support in the neonatal period, risk factors should be identified and preventive measures should be developed. Preventive strategies, raising awareness of families, careful and meticulous attitudes especially during high season might reduce the incidence and hospitalizations of the infants.

Key Words: Neonatal intensive care unit, Respiratory syncytial virus, Respiratory tract infections

ÖZ

Amaç: Son yıllarda virusların mutipleks gerçek zamanlı polimeraz zincir reaksiyonu ile belirlenmesi, enfeksiyonlara neden olan viral ajanların izole edilmesine imkan sağlamıştır.

Bu çalışmanın birincil amacı yenidoğan yoğun bakım ünitesinde akut alt solunum yolu enfeksiyonuna neden olan solunum yolu viruslarının dağılımını belirlemek ve RSV ve RSV dışı enfeksiyonu olan bebeklerin demografik ve klinik özelliklerini karşılaştırmaktır. İkincil amacı ise solunum desteği gerektiren risk faktörlerinin belirlenmesidir.

Gereç ve Yöntemler: Bu geriye dönük gözlemsel çalışma Ocak 2016 ile Haziran 2019 yılları arasında yapıldı. Bebekler RSV ve RSV dışı olmak üzere iki gruba ayrılarak karşılaştırıldı. Solunum desteği gerektiren hastalar (invaziv ve non-invaziv ventilasyon) için risk faktörleri belirlendi.

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Bulgular: Viral alt solunum yolu enfeksiyonu tanısı ile 243 bebek hastaneye yatırıldı ve bu bebeklerden viral etken saptanan 119'u çalışmaya dahil edildi. En sık saptanan virüs RSV'di (n=93, %78). RSV ve RSV dışı gruplardaki bebeklerin demografik özellikleri postnatal yaş dışında benzerdi. Bebekler en sık Aralık ve Şubat ayları arasında hastaneye yatırıldı. Solunum desteği gerektiren risk faktörlerini belirlemek için yapılan multivariate analizinde apne ve RSV risk faktörü olarak bulundu.

Sonuç: Yenidoğan döneminde RSV ile ilişkili akut alt solunum yolu enfeksiyonları en sık görülen ve daha fazla solunum desteği gerektirdiğinden, risk faktörleri tanımlanmalı ve önleyici yöntemler geliştirilmelidir. Önleyici stratejiler, ailelerin farkındalıklarının artırılması, özellikle sezonda dikkatli ve titiz davranışlarla bebeklerde enfeksiyonların görülme sıklığı ve hastaneye yatışlar azalabilir.

Anahtar Sözcükler: Yenidoğan yoğun bakım ünitesi, Respiratory syncytial virüs, Solunum yolu enfeksiyonları

INTRODUCTION

Newborns, especially premature infants, are highly susceptible to infections due to their inadequate natural and adaptive immune systems (1). The etiological origin of viral respiratory infections is often unknown. The causative virus can be transmitted vertically through the mother in utero as well as from the the environment or mother after birth. Although viral acute lower respiratory tract infections (LRTI) are usually mild in neonates, they can cause significant morbidity and mortality especially in premature infants. Viral lower respiratory infections in early life have been associated with wheezing attacks and asthma later in life (2).

In recent years, detection of viruses by multiplex real time polymerase chain reaction (RT-PCR) allowed the isolation of causative viral agents for LRTI (3). The most common causes of acute viral LRTI infancy and early childhood are respiratory syncytial virus (RSV), human rhinovirus (hRV), human para-influenza virus (hPIV), human influenza virus (hIV) and human metapneumovirus (hMPV) in infancy and early childhood (4). RSV is the most common cause of LRTI in infancy and early childhood and also it is the second most common cause of infant mortality after neonatal period (5). RSV season lasts from October to March (most commonly in January-February) in temperate climates and all year round in tropical climates. The prevalence of RSV was found to be 37.9% in patients with acute viral LRTI within the first 24 months and 19.6% for hospitalized newborns in our country (6,7).

The primary objectives of this study were to determine the distribution of causative respiratory viruses in acute LRTI in the neonatal intensive care unit (NICU) and to compare the demographic and clinical characteristics of infants with RSV and non-RSV infections. The secondary aim was to determine risk factors requiring respiratory support.

MATERIALS and METHODS

This retrospective observational study was conducted between January 2016 and June 2019 at the Health Sciences University, Ankara Children's Hematology Oncology Research Hospital. The study protocol was approved by the local Ethic Committee (no: 2019-111/19.04.2019).

Premature infants, postmenstrual age up to 44 gestation weeks, and term infants, postnatal age up to 28 days, diagnosed with acute LRTI and hospitalized in the NICU were included in this study. In our country, palivizumab prophylaxis is applied to premature babies born before 29 weeks during RSV season according to the guideline of the Turkish Neonatal Society (8). The infants with major congenital abnormalities, hemodynamically significant congenital heart diseases, bronchopulmonary dysplasia, bacterial growth in the blood culture and also patients in which no viral agent could be determined in the nasopharyngeal swab were excluded. The infants with at least one of the following below were hospitalized: 1. Respiratory distress (respiratory rate above 60/minute, wheezing, cough, intercostal/subcostal retractions, apnea) and hypoxia (oxygen saturation <90 % measured by pulse oximetry in room air), 2. Impaired circulation (tachycardia, low blood pressure, prolonged capillary refill time), 3. Respiratory distress accompanied by feeding difficulty.

The infant's birth weight, gestational age, sex, maternal age, mode of delivery, breastfeeding history and presence of respiratory tract infection in the family were recorded. The findings of physical examination, signs of respiratory failure (tachypnea, tachycardia, nasal flaring, subcostal and intercostal retractions, groaning), laboratory findings (hemogram, peripheral blood smear, blood gas analysis, C-reactive protein (CRP)), radiological examinations (chest X-ray), and monitoring in the NICU (oxygen therapy, respiratory support (non-invasive and invasive ventilation), and duration of respiratory support) were evaluated.

The nasopharyngeal swab samples were taken for multiplex RT-PCR analysis within one hour following hospitalization; and RSV, hRV, hIV, hPIV, hMPV, human Boca virus (hBV), corona virus (CV), entero virus (EV), and adeno virus (AV) PCR were studied. RNA extraction was done with a Total Nucleic Acid Isolation Kit in Magnapure LC 2.0 isolation machine (Roche, Germany) or with EZ1 virus Mini Kit and Qiasymphony Virus/Bacteria Mini Kit in EZ1 Advanced XL and Qiasymphony isolation machine (Qiagen Gmbh, Hilden, Germany). Viral RNA was searched by

using either an "in-house" revers transcriptase real-time PCR (rt RT-PCR) protocol provided by the Center for Disease Control or Qiagen artus Infl/H1 LC/RC RT-PCR Commercial Kit (Qiagen artus GmbH, QIAGEN Strasse 1, D-40724 Hilden, Germany). In house Real-time RT- PCR was performed on ABI 7500. The 25 ml PCR mixture contained 5 ml of extracted RNA, 1 ml each of forward and reverse primers, 1 ml probe, 0.5 ml SuperScript III RT/ Platinium Taq mix, 12.5 ml of 2X Master mix, and 4 ml nuclease-free water. RT-PCR amplification conditions were as follows; reverse transcription at 50 °C for 30 min, Taq inhibitor activation 95°C for 2 min and 45 cycles at 95°C for 15 sec, 55°C for 30 sec.

The infants were divided into two groups as RSV and non-RSV, and compared. The identification of RSV alone or together with other viruses was defined in the RSV group. Additionally, risk factors for respiratory support by means of invasive/non-invasive ventilation were determined.

IBM SPSS Statistics 17.0 programme was used for statistical analysis. Shapiro-Wilk test was used to determine whether the variables normally distributed. We described the variables with non normal distribution as median (minimum-maximum), and the variables with normal distribution as mean ± SD (Standard Deviation). Chi-square test was used for the analysis of categorical variables, and Mann Whitney U test was used for the analysis of numerical data with non normal distribution. The risk factors for respiratory support requirement was determined by multivariate logistic regression analysis. A p value less than 0.05 was considered statistically significant.

RESULTS

Between January 2016 and June 2019, a total of 2462 patients were hospitalized in the NICU. Among them, 243 infants were hospitalized with the diagnosis of acute viral LRTI and a causative viral agent could be detected in 187 (77%) of them. Medical records of 68 patients couldn't be reached; so a total of 119 infants' data were recorded and used for the study. RSV was the most common isolated virus (n=93, 78%) among viral LRTI. The demographic characteristics of infants in RSV and non-RSV groups were similar, except postnatal age (Table I). The age of hospitalization in the RSV group was found to be smaller compared to non-RSV group (p=0.045) (Table I).

All causative viral agents and their distribution were shown on Table II. The frequency of hIV was 2.5% in our study, but oseltamivir treatment was given to 12% of cases due to severe clinical findings. The highest rates of hospitalization due to acute viral LRTI were determined in between December and February (Figure 1).

The infants' hemoglobin (p=0.013) and CRP levels (p=0.033) in the RSV group were lower than non-RSV group on admission. Among the infants' complaints and findings, cough (p=0.00), retractions (p=0.023), crackles (p=0.018) and rhonchi (p=0.024) were higher in the RSV group (Table III).

The frequency of apnea (p=0.003), immature to total neutrophil (I / T) ratio (p=0.006), retractions (p=0.019), crackles (p= 0.00), RSV PCR positivity (p=0.041) and length of hospital stay (p=0.00) were found to be higher in the infants requiring respiratory support (Table IV). In multivariate analysis, apnea and RSV PCR positivity were found to be risk factors for respiratory support requirement (Table V). During the study, only one infant dead in the RSV group, who had been followed on ECMO at another center. He was a refugee, born at 27-weeks gestational age and discharged healthy on the newborn period. This infant was not given palivizumab prophylaxis although recommended.

Table I: Demographic characteristics of infants with RSV and non-RSV viral LRTI.

Characteristics	Total (n=119)	RSV (+) (n=93)	RSV (-) (n=26)	p
Birth weight (g), median (range)	3020 (1140-4200)	3080 (1350-4200)	2730 (1150-4200)	0.210
Gestational age (weeks), median (range)	38 (27-42)	38 (27-42)	36 (27-41)	0.075
Gender, n (%)				
Female	44 (37)	36 (39)	9 (35)	0.137
Male	74 (62)	57 (61)	17 (65)	
Delivery, n (%)				
Vaginal	44 (37)	36 (39)	8 (31)	0.458
Cesarean	75 (63)	57 (61)	18 (69)	
Maternal age, (year) median (range)	28 (16-44)	28 (14-44)	28 (18-37)	0.859
Other child in household, n (%)	96 (81)	74 (80)	22 (85)	0.191
Bresastfeeding, n (%)	110 (92)	89 (96)	21 (92)	0.110
History of infection at home, n (%)	71 (60)	56 (60)	15 (58)	0.817
Postnatal age, (day) median (range)	24 (7-112)	24 (7-78)	29 (7-112)	0.045

** **LRTI:** Lower respiratory tract infection, **RSV:** Respiratory syncytial virus.*

Table II: Viral agents identified in the infant's nasopharyngeal swab samples.

Agents	n (%)
Respiratory syncytial virus	93 (78.0)
Human rhinovirus	12 (10.0)
Human coronavirus	4 (3.0)
Parainfluenza	3 (2.5)
Human influenza virus A/B	3 (2.5)
Metapneumovirus	2 (2.0)
Enterovirus	1 (1.0)
Adenovirus	1 (1.0)
Co-infection: RSV + human Rhinovirus (2) RSV + parainfluenza (1)	3

***RSV:** Respiratory syncytial virus.*

Table III: Clinical and laboratory findings of RSV and non-RSV infants with viral LRTI.

Clinical and laboratory findings	Total (n=119)	RSV (+) (n=93)	RSV (-) (n=26)	p
Clinical features on admission				
Fever (°C), median (range)	36.5 (35.2-38.4)	36.5 (36-38.4)	36.6 (35.2-38.4)	0.160
Cough,*	110 (92)	91 (98)	19 (73)	<0.00
Rhinorrhea, *	24 (20)	19 (20)	5 (19)	0.893
Nasal congestion, *	46 (39)	38 (41)	8 (31)	0.350
Apnea, *	12 (10)	7 (7)	5 (19)	0.080
Unrest, *	47 (39)	31 (33)	16 (61)	0.009
Laboratory findings, median (range)				
Hemoglobin (g/dl)	12.7 (6.5-19.3)	12.7(8.4-17.4)	12.9 (6.5-19.3)	0.013
White blood cell count (/mm³)	9000 (1100-35500)	8850 (1200-35500)	9600 (1100-28500)	0.409
Lymphocyte percentage	60 (6-90)	62(6-90)	58 (16-86)	0.825
CRP (mg/dl)	0.43 (0-15.5)	0.35(0.01-10.6)	0.45 (0-15.5)	0.033
I/T	0.1 (0-0.75)	0.09(0-0.75)	0.15 (0-0.40)	0.964
Chest X-ray findings, *	82 (72)	65 (71)	17 (77)	0.535
Infiltration	36 (32)	28 (30)	8 (36)	0.591
Consolidation	12 (10)	12 (13)	0 (0)	0.730
Hyperinflation	11 (10)	11 (12)	0 (0)	0.088
Atelectasis	5 (4)	5 (5)	0 (0)	0.263
Respiratory system findings, *				
Respiration rate	52 (32-100)	52 (32-88)	54 (40-100)	0.240
Grunting	6 (5)	4 (4)	2 (8)	0.485
Retractions	69 (58)	59 (63)	10 (38)	0.023
Rales	61 (51)	53 (57)	8 (31)	0.018
Rhonchi	35 (29)	32 (34)	3 (11)	0.024
Prolonged expirium	56 (47)	48 (52)	8 (31)	0.060
Oxygen requirement, * (only)	66 (55)	47 (50)	19 (73)	0.325
Respiratory support, *				
HFNC	20 (17)	18 (19)	2 (8)	0.160
n-CPAP/NIPPV	46 (39)	40 (43)	6 (23)	0.065
Invasive ventilation	14 (12)	13 (14)	1 (4)	0.156
Treatment, *				
Salbutamol	51 (43)	44 (47)	7 (27)	0.063
Hypertonic salin	16 (13)	16 (17)	0 (0)	0.023
Oseltamivir	14 (12)	10 (11)	4 (15)	0.815
Antibiotic	116 (97)	90 (97)	26 (100)	0.354
Duration of antibiotic use, (day), median (range)	8 (0-17)	9 (0-17)	8 (0-17)	0.172
Duration of hospitalization, (day) median (range)	8 (3-27)	8 (4-27)	8 (3-21)	0.694

***n%, **CRP**:C-reactive protein, **I /T ratio**: Immature total neutrophil ratio, **LRTI**: Lower respiratory tract infection, **RSV:** Respiratory syncytial virus, **HFNC**: High flow nasal cannula, **n-CPAP**: Nasal continous positive airway pressure, **NIPPV**: Nasal intermittent positive pressure ventilation.*

Table IV: Characteristics of viral LRTIs with and without respiratory support.

Characteristics	Respiratory support (+), (n=53)	Respiratory support (-), (n=66)	p
Birth weight, (g) median (range)	2920 (1360-4200)	3085 (1140-4200)	0.787
Gestational age, (week), median (range)	38 (30-41)	38 (27-42)	0.532
Gender, *			
Female	21 (40)	23 (35)	0.596
Male	32 (60)	42 (64)	
Clinical features on admission			
Fever (°C), median (range)	36.6 (35.2-38.4)	36.5 (36-38.4)	0.459
Cough, *	50 (94)	54 (91)	0.477
Apnea, *	10 (19)	2 (3)	0.003
Unrest, *	24 (45)	23 (35)	0.247
Laboratory findings, median (range)			
Hemoglobin, (g/dl)	12.9 (6.5-19.3)	12.7 (8.4-17.4)	0.363
White blood cell count, (/mm³)	9600 (1100-28500)	8850 (1200-35500)	0.274
Lymphocyte percentage	58 (16-86)	62 (6-90)	0.160
CRP, (mg/dl)	0.45 (0-15.5)	0.35 (0.01-10.6)	0.518
I/T ratio	0.15 (0-0.4)	0.09 (0-0.75)	0.006
Abnormal chest X-ray findings, *	36 (72)	46 (72)	0.988
Respiratory system findings			
Respiration, rate /min	54 (40-100)	52 (32-88)	0.146
Grunting, *	4 (7)	2 (3)	0.263
Retraction, *	37 (70)	32 (48)	0.019
Rales, *	37 (70)	24 (36)	0.00
Rhonchi, *	17 (32)	18 (27)	0.568
Prolonged expirium, *	28 (53)	28 (42)	0.258
RSV (+), *	45 (85)	47 (71)	0.041
Duration of hospitalization, (day) median (range)	10 (5-27)	7 (3-14)	< 0.00

***n (%), **CRP:** C-reactive protein, **I /T ratio:** Immature total neutrophil ratio, **LRTI:** Lower respiratory tract infection, **RSV:** Respiratory syncytial virus.*

Table V: Risk factors for respiratory support.

Risk factor	Univariate			Multivariate		
	OR	95%CI	p	OR	95 % CI	p
Birth weight, (g)	1	0.999-1.000	0.637			
Gestational age, (week)	0.976	0.868-1.096	0.678			
Gender	0.771	0.439-1.353	0.365			
Apnea	7.44	1.554-35.649	0.012	12.969	2.277-73.848	0.004
RSV	2.657	1.020-6.919	0.045	4.384	1.380-13.931	0.012

DISCUSSION

In this study, we evaluated the demographic and clinical characteristics of the infants hospitalized with viral LRTI in the NICU. RSV (78%) and hRV (10%) were the most common isolated viral agents and RSV peak was observed during January and February. Demographic characteristics of the infants were similar between RSV and non-RSV groups. The age at hospitalization was lower in the RSV group. RSV PCR positivity detected more in patients who required respiratory support and also length of hospital stay was longer in this group. The presence of apnea and isolation of RSV were found to be risk factors for respiratory support requirement in patients with acute viral LRTI.

The gold standard for the detection of respiratory viruses is RT-PCR, with a sensitivity of 93-100% and a specificity of 64-100% (9). The rate of viral agent detection in newborns with viral LRTI was found to be 69-80% (10-12). We identified viral causative agent in 77% of our patients by RT-PCR, similar to the literature. It has been shown that identification of viral agents by RT-PCR can decrease unnecessary antibiotic therapy, shorten the length of hospital stay and isolation periods of the patients (13). RSV is the most common identified pathogen in young children with LRTI and an important cause of hospital admissions (14). In the NICUs, RSV is the most frequently detected agent in acute viral LRTI (19.5-47%) (7,10-12). We identified RSV 38% among all of the viral LRTI and RSV comprised 78% of all isolated agents. Alan et al. conducted a multicenter study in our

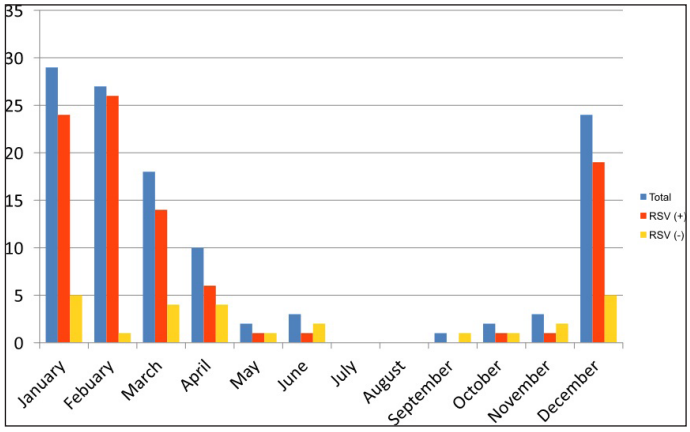

Figure 1: Distribution of viral agents by months.

country and reported that 19.5% of viral LRTI were caused by RSV in the NICUs (7). But, the study was terminated in March. We propose that, the low ratio of RSV compared to our study might be related to the isolation of RSV by different techniques (monoclonal antibody) or heterogeneity between centers.

In this study, hRV (10%) was the second most common detected virus. The incidence of hRV was found to be 12.5-19.1% in the newborns and infants younger than 1 year of age hospitalized due to LRTI (10,15-17). Recent studies have shown that the frequency of hRV in hospitalized children with viral LRTI is higher than RSV (18,19). The prevalence of influenza in neonatal period was reported to be quite low (10-12). Although the frequency of influenza was 2.5% in our study, 12% of the cases were treated with oseltamivir due to severe clinical findings. Influenza infections are thought to be uncommon in neonates, due to maternal antibodies, breastfeeding and low contact with virus-infected adults (20,21). Vij et al. (22) detected hIV in 3 premature infants in 2009 pandemics, but they did not initiate oseltamivir and PCR was negative after an average of 6 days. The low mortality rate of influenza infections in neonates may be due to the awareness of the employees, early diagnosis and preventive approaches (23). In neonates, oseltamivir treatment may be more appropriate for only in pandemics or in patients with severe clinical findings.

The frequency of viral LRTI was highest between December and April. RSV was most commonly seen in January and February. The seasonal distribution of RSV in the world varies according to the geographical region. A recent study from Greece, with similar climate as our country, evaluated the distribution of RSV infections during 12 years (24). RSV season was between December and April, and peaked in February similar to our results (24). In a multicenter study conducted by the Turkish Society of Neonatology between 2008 and 2010, RSV infection was observed between October and March and peaked between January and March (25). According to our results comprising four years, it would be more appropriate to initiate palivizumab prophylaxis late and extend it to April, since RSV is more frequent in April than November. Prospective multicenter studies should be performed as seasonal distributions of viral agents might be changed over years.

In the literature, it has been shown that the age at hospitalization is younger in RSV group compared to the non-RSV group (11,12). But in Cho et al.'s study it was similar between the groups (10). We found that infants' age at admission was lower in the RSV group. However, birth weight and gestational age did not differ between the groups (10-12). This finding might be explained by protection of the preterm and risky infants by palivizumab prophylaxis.

We identified that infants in RSV and non-RSV groups had similar complaints on admission; and clinical and laboratory findings and length of hospital stay did not differ between groups. Cough, dyspnea, crackles and rhonchi were more common in RSV group. Similarly, Çelik et al. (12) showed that cyanosis, tachycardia, tachypnea, rhonchi, expiratory lengthening and crackles were more common in the RSV group. In another study, Cho et al. (10) reported that pneumonia, oxygen demand, consolidation in the right upper lobe, and longer hospital stay were common in the RSV group. Other studies have shown that the clinical manifestations of RSV infection under 5 years are more severe than the other viral respiratory infections (10,26,27).

Requirement of invasive mechanical ventilation was 12% and HFNC was applied to 17% of the patients in this study. Similar to our results, it was shown that approximately 11% of the patients needed invasive respiratory support (7,11). In Cho et al.'s (10) study, 4.4% of the patients needed invasive respiratory support. In a recent multicenter study, HFNC failure in neonates with respiratory distress was higher than CPAP; even so comparative studies reported that CPAP was superior to HFNC and HFNC was superior to oxygen therapy in the infants diagnosed with bronchiolitis (28-30). We suggest that, in neonates with LRTI, firstly oxygen therapy, but if patient's respiratory distress persists, then HFNC prior to CPAP and lastly invasive mechanical ventilation might be applied. We determined that the presence of apnea and RSV infections were found to be risk factors for respiratory support requirement in the infants with viral LRTI. There are conflicting results about prematurity and the severity of acute viral LRTI in the literature; but we showed that prematurity was not a risk factor for the severity of LRTI by means of respiratory support, and this might be related to the application of palivizumab prophylaxis to infants born before 29 weeks gestational age in our country (8, 31-34).

The first limitation of this study was the small sample size as it was a single-center study, and so could not represent the whole country. Second, because of the retrospective nature of the study, we could not obtain all the recorded clinical data

of some infants who had PCR proven-viral LRTI. The risk factors and epidemiological features of viral LRTI need to be demonstrated by multicentre prospective studies.

In conclusion, RSV is the most common detected agent for viral LRTI in the newborn infants. Since RSV-associated LRTI requires more frequent and more respiratory support in the neonatal period, risk factors should be identified and preventive measures should be developed. RSV vaccine may be a solution, and palivizumab prophylaxis should be more widely and effectively used. Preventive strategies, raising awareness of families, careful and meticulous attitudes especially during high season might reduce the incidence of viral LRTIs and hospitalizations of the infants.

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