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RESEARCH ARTICLE/KLİNİK ÇALIŞMA

FLORA 2020;25(1):69-75 • doi: 10.5578/flora.68758

Viral Pathogens and Seasonal Distribution in Respiratory Tract Infections

Solunum Yolu İnfeksiyonlarında Viral Patojenler ve Mevsimsel Dağılımları

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Cite this article as: *Maçin S, Fındık D. Viral pathogens and seasonal distribution in respiratory tract infections. FLORA 2020;25(1):69-75.*

ABSTRACT

Introduction: *Respiratory tract infections cause significant morbidity and mortality. Viruses are one of the leading causes for respiratory tract infections all over the world. The aim of this study was to determine viral pathogens in respiratory tract infections and to investigate their seasonal distribution.*

Materials and Methods: *The seasonal distribution of viral agents in nasopharyngeal swab specimens taken from 1999 patients with a prediagnosis of respiratory tract infection between January 1, 2014 and December 31, 2018 was investigated by multiplex real-time polymerase chain reaction method.*

Results: *2034 viruses were detected in 1999 nasopharyngeal swab specimens. As the most common viral agents; rhinovirus (23.5%), influenza virus (18.1%) and respiratory syncytial virus (17.9%) were determined. Only one viral agent was detected in 1563 (78.18%) of all patients. Two (19.1%) viruses in 381 patients, 3 (2.8%) viruses in 57 patients and 4 (0.4%) viruses in 8 patients were detected. Respiratory syncytial virus (17.9%) was the most common viral agent in winter, and rhinovirus (6%) was the most common viral agent in spring. The most common viral agents were found in winter (42.6%) and the lowest in summer (6.9%).*

Conclusion: *The detection of multiple viral agents in a single study by polymerase chain reaction in respiratory tract infections will prevent unnecessary use of antibiotics in patients. With necessary precautions and symptomatic treatments, the duration and severity of symptoms will be reduced and possible complications and transmission will be prevented.*

Key Words: *Multiplex polymerase chain reaction; Respiratory tract infections; Season; Virus*

Received/Geliş Tarihi: 10/05/2019 - Accepted/Kabul Ediliş Tarihi: 18/10/2019

ÖZ

Solunum Yolu İnfeksiyonlarında Viral Patojenler ve Mevsimsel Dağılımları

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Giriş: *Solunum yolu infeksiyonları, önemli derecede morbidite ve mortaliteye neden olur. Tüm dünyada virüsler, önde gelen solunum yolu infeksiyonu etkenlerindendir. Bu çalışmada, solunum yolu infeksiyonlarında viral patojenlerin saptanması ve mevsimsel dağılımının araştırılması amaçlanmıştır.*

Materyal ve Metod: *Çalışmada, 1 Ocak 2014-31 Aralık 2018 tarihleri arasında, solunum yolu infeksiyonu ön tanısı olan, 1999 hastadan gönderilen nazofarengeal sürüntü örneklerinde saptanan viral etkenlerin mevsimsel dağılımı retrospektif olarak incelendi. Multipleks gerçek-zamanlı polimeraz zincir reaksiyonu yöntemi kullanılarak viral etkenler saptandı.*

Bulgular: *Toplam 1999 hasta örneğinde 2034 virüs saptanmıştır. En sık viral etkenler; rinovirüs (%23.5), influenza virüs (%18.1) ve respiratuvar sinsityal virüs (%17.9) olarak tespit edilmiştir. Hastaların 1563 (%78.2)'ünde tek bir viral etken saptanmıştır. Hastaların 381'inde 2 (%19.1) virüs, 57'sinde 3 (%2.8) virüs, 8'inde ise 4 (%0.4) virüs birden saptanmıştır. En sık viral etkenin; kış aylarında respiratuvar sinsityal virüs (%17.9), ilkbahar aylarında ise rinovirüs (%6) olduğu tespit edilmiştir. Viral etkenler en sık kış aylarında (%42.6), en az ise yaz aylarında (%6.9) saptanmıştır.*

Sonuç: *Solunum yolu infeksiyonlarında, polimeraz zincir reaksiyonu ile birden fazla viral etkenin tek bir çalışmada saptanacak olması hastalarda gereksiz antibiyotik kullanımını önleyecektir. Gerekli önlemlerin ve semptomatik tedavilerin yapılmasıyla semptomların süresi ve şiddeti azalacak, olası komplikasyonlar engellenerek bulaş önlenmiş olacaktır.*

Anahtar Kelimeler: *Mevsim; Solunum yolu infeksiyonları, Virüs; Multipleks polimeraz zincir reaksiyonu*

INTRODUCTION

Respiratory tract infections are very common and can be seen as an important public health problem as they can be epidemic in humans[1]. Viral respiratory infections are the most common infections in all age groups, especially in children, and are the most important cause of mortality. Approximately 80% of respiratory tract infections are caused by viruses[2].

Most viruses are transmitted through the droplet and primarily cause infection in the airway epithelium. Viruses can cause respiratory insufficiency, particularly in childhood, by causing diseases such as upper respiratory tract infections, bronchiolitis, pneumonia, chronic lung disease and triggering of acute asthma attack[3-6]. While influenza A/B/C (FLU), human parainfluenza virus (HPIV) and human respiratory syncytial virus (HRSV A/B) usually cause epidemics, adenovirus, coronavirus and rhinovirus (RV) are the causes of endemic infections. In addition to known factors, new generation respiratory viruses that cause acute respiratory insufficiency such as human metapneumovirus (HMPV A/B), human coronavirus (HCoV), adenovirus (HAdV), human parechovirus (HPeV), human bocavirus (HBoV) etc. have been identified in recent years[7].

In the outpatient treatment of viral upper respiratory tract infections (URI); analgesics, antipyretics, nasal and systemic decongestants, antihistamines, antitussive and/or expectorants and antibiotics are frequently prescribed. Although it has mild and self-healing properties, URI has a high treatment cost due to both prescribed drugs for symptomatic treatment and inappropriate use of antibiotics[8]. In the prevention and treatment of viral upper respiratory tract infections, it is very important to raise awareness of the public and physicians. The aim of this study was to investigate the seasonal distribution of viral upper respiratory tract infection agents in our hospital.

MATERIALS and METHODS

Collection of Samples

The seasonal distribution of viral agents in nasopharyngeal swabs collected between 1 January 2014 and 31 December 2018 in Selcuk Univer-

sity Faculty of Medicine Hospital was examined retrospectively. The nasopharyngeal swab specimens were collected with the FLOQSwabs (Brescia, Italy) bar in UTM-RT (USA) transport solution.

Quantitative PCR Method

DNA isolation: DNA isolation was made by using the Qiagen-EZ1 Virus Mini kit (Hilden, Germany) in accordance with the company recommendations.

Quantitative PCR: Primary mix was prepared in 8 separate wells for PCR by using Fast track diagnostics (Luxembourg) kit. The total mix quantity was adjusted to 15 µL per well. 10 µL of isolated genome was placed into each well. Gene amplification was performed on Qiagen Rotor-gene Q device (Hilden, Germany).

The kit contains; HRSV, HMPV, HCoV, HPIV, HAdV, HBoV, RV, cytomegalovirus (CMV), FLU, enterovirus (EV), HPeV as viral pathogen, *Streptococcus pneumoniae*, *Moraxella catarhalis*, *Bordetella pertussis*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Legionella* spp., *Salmonella* spp. and *Chlamydia pneumoniae* as bacterial pathogen and only *Pneumocystis jirovecii* as fungal pathogen.

Statistical Analysis

All analyzes were performed using SPSS 17.0 package program. The Chi-square test (Fisher Exact Test/Exact Test) was used in order to analyze the relationship between two categorical variables. $p< 0.05$ was considered statistically significant.

Ethics Committee Approval

Permission was obtained from Selcuk University Faculty of Medicine Non-Interventional Ethics Committee with the decision dated 17.11.2015 and numbered 2015/301.

RESULTS

In this study, 1999 nasopharyngeal swab specimens were evaluated retrospectively for viral agents causing respiratory tract infection (HRSV, HMPV, HCOV, HPIV, HAdV, HBoV, RV, CMV, FLU, EV, HPeV). Nasopharyngeal swab samples were sent to our laboratory from pediatric allergy and immunology service, pediatric infectious diseases service, infectious diseases service, pediatric oncology service, Neonatal intensive care unit, pediatric outpatient clinic and pediatric emergency polyclinics.

When the age distribution of the patients with viral agents was examined; 68.2% of the patients were found to be pediatric patients. Viral agents were found most frequently and statistically significantly ($p< 0.05$) in patients under two years of age (44%). Age distribution of patients is given in Table 1.

From the 1999 samples, a total of 2034 viral agents were detected in five years period. 1021 patients (51.1%) were males and 978 (48.9%) were females. The number and seasonal distribution of viruses determined from nasopharyngeal swab specimens of patients are given in Table 2. 1061 (53.1%) of the samples were accepted to our laboratory during winter. Viral agents were found to be the most in winter (42.6%) and the lowest in summer (6.9%) in our study.

Of 1999 samples, only one viral agent was found in 1563 (78.2%). In 381 samples, 2 (19.1%) viruses, in 57 samples 3 (2.8%) viruses and in 8 samples 4 (0.4%) viruses were detected at the same time. The distribution of single or

Table 1. The age groups of viral agent-detected patients

Years	< 2 years n (%)	3-4 years n (%)	5-18 years n (%)	> 18 years n (%)	Total
2014	143 (56.5)	43 (17)	40 (15.8)	27 (10.7)	253
2015	83 (48.2)	5 (2.9)	19 (11.2)	65 (37.7)	172
2016	78 (32.6)	9 (3.8)	33 (13.8)	119 (49.8)	239
2017	214 (43.7)	49 (10)	94 (19.2)	133 (27.1)	490
2018	361 (42.7)	59 (7)	134 (15.9)	291 (34.4)	845
Total	879 (44.0)	165 (8.3)	320 (16)	635 (31.8)	1999

Table 2. The number and seasonal distribution of the detected viruses

	Winter	Spring	Summer	Autumn	Total
Number of samples	1061	468	142	328	1999
Number of detected viruses	866	463	141	564	2034

Table 3. The distribution of single or multiple detection rates of viral agents by years

Years	Single virus n (%)	Two viruses n (%)	Three viruses n (%)	Four viruses n (%)	Total
2014	197 (77.8)	50 (19.8)	6 (2.4)	0	253
2015	148 (86)	20 (11.6)	4 (2.4)	0	172
2016	215 (89.8)	23 (9.5)	1 (0.7)	0	239
2017	325 (65.4)	138 (29,2)	21 (4,9)	6 (1.2)	490
2018	678 (79.3)	150 (17.6)	25 (2.9)	2 (0.2)	845
Total	1563 (78.2)	381 (19.1)	57 (2.8)	8 (0.4)	1999

Table 4. Distribution of viruses between 2014-2018 according to seasons

Viruses	Winter n (%)	Spring n (%)	Summer n (%)	Autumn n (%)	Total n (%)
HRSV A/B	258 (12.7)	93 (4.6)	4 (0.2)	9 (0.4)	364 (17.9)
HMPV A/B	44 (2.2)	38 (1.9)	2 (0.1)	0	84 (4.1)
HCoV	78 (3.9)	42 (2.1)	16 (0.8)	42 (2.1)	178 (8.8)
HPIV	35 (1.8)	20 (0.1)	28 (1.4)	32 (1.6)	115 (5.7)
HAdV	17 (0.8)	21 (1.0)	16 (0.8)	11 (0.5)	65 (3.2)
HBoV	19 (0.9)	9 (0.4)	5 (0.3)	4 (0.2)	37 (1.8)
RV	149 (7.3)	123 (6.0)	58 (2.9)	149 (7.3)	479 (23.5)
CMV	34 (1.7)	25 (1.2)	8 (0.4)	7 (0.3)	74 (3.6)
FLU A/B/C	231 (11.4)	90 (4.4)	0	48 (2.4)	369 (18.1)
EV	0	1 (0.1)	2 (0.1)	2 (0.1)	5 (0.3)
HPeV	1 (0.1)	1 (0.1)	2 (0.1)	0	4 (0.2)
Total	866 (42.6)	463 (22.8)	141 (7.0)	564 (27.8)	2034 (100)

HRSV: Human respiratory syncytial virus, HMPV: Human metapneumovirus, HCoV: Human coronavirus, HPIV: Human parainfluenza virus, HAdV: Adenovirus, HBoV: Human bocavirus, RV: Rhinovirus, CMV: Cytomegalovirus, FLU: Influenza virus, EV: Enterovirus, HPeV: Human parechovirus.

multiple detection rates of viral agents by years is given in Table 3.

HRSV A/B virus (17.9%) was the most common viral agent in the form of single or co-infection in winter (December-January-February). In spring (March-April-may), rhinovirus (6%) was the most common agent. Summer (June-July-August) was the period of time when the patient samples were less and the least viral agents were detected. Rhinovirus (2.9%) was the most common cause of upper respiratory tract infections in summer. In autumn (September-October-November), rhinovirus was the most common (7.4%) agent and HMPV A/B and HPeV were not detected in any sample. When the total of 5 years evaluation is examined; the most common viral agent was rhinovirus (%23.5) while the least (0.2%) was HPeV. The distribution of viruses in our study according to seasons is given in Table 4.

DISCUSSION

Upper respiratory tract infections are one of the major causes of morbidity and mortality worldwide[9]. Malnutrition, low birth weight, passive smoking, low socio-economic status, overcrowded environment, old age, immunodeficiency and HIV infection are risk the factors for upper respiratory tract infections. Viral respiratory infections are the most common cause of hospitalization especially in infants and young children[10].

Patient age is always an important factor in predicting the causative microorganism. It is important to know the age distributions of respiratory tract viruses. When our cases were classified as under 2 years of age, 3-4 years, 5-18 years and over 18 years of age, viral factor detection rates were found to be different, the infection rate in children under 5 years of age was found to be 52.3% and this value was statistically significant ($p< 0.05$). In the United States, researchers have found the rate of viral agents as 61% in children under 5 years of age[11].

Molecular methods for respiratory pathogens have facilitated the detection of viruses in the respiratory tract of patients. In Turkey, at least one viral agent detection rate was found between 41.8% and 83.3%. In our study, a single viral agent was detected in 1563 (78.2%) of the patients, and this is consistent with the data of our country. The rates consistent with our study have also been reported from different countries: in studies from Sweden, Japan and France; at least one viral factor has been reported in 48%, 85.3% and 88.7% respectively[15-17].

Cilla et al. have found at least one virus in 66.9% of the samples, and the most frequently detected virus was RSV. They have also reported that children with multiple virus infections had to be hospitalized more often than children with single virus infections[18]. Although there are several studies showing that patients with more than one viral agent have a more severe course, there are also studies that found it to be ineffective or milder[19-22].

In the temperate climate zone, there are typical seasonal distributions of common respiratory tract viruses. RSV and influenza viruses have been reported to increase in winter[16]. Influenza virus epidemics are seen in winter and autumn in temperate regions, including our country. These epidemics cause 3-5 million diseases and 250-500 thousand deaths all over the world[23]. RSV causes outbreaks in temperate zones at the beginning of winter and spring, but its activity may vary locally[24]. In studies performed in our country; RSV has been reported to be seen between November and April, especially in the months of December and January[25,26]. In our study, RSV and influenza viruses were found most frequently in winter, in consistent with these data. In a study conducted in Japan, RSV has been found more at the end of the year, and influenza A has been found frequently between January and March[27].

In our study, rhinovirus was seen all year round, but it displayed an increase in winter and spring months. HMPV had a similar seasonal distribution with RSV. Parainfluenza virus was also found all year round. HCoV was very high during the winter but it was also be determined all year round. CMV and HAdV caused respiratory infections all around the year. Bayrakdar et al., have identified rhinoviruses and parainfluenza all around the year in accordance with our study[28].

RSV may cause wheezing in lower respiratory tract infections, especially in young children whereas rhinovirus has been shown to be as a cause of asthma exacerbations in older children. Sancakli et al. have identified rhinovirus (26.4%) and RSV (10.3%) as the most common viral agents[13]. In our study, the most common viral agents were rhinovirus (23.5%), influenza (18.1%) and RSV (17.9%). Long et al., have detected, influenza virus (25%), rhinovirus (40%) and MPV (10%) as the most common viral agents[15]. In a study conducted in France, RSV (39.5%), rhinovirus (24.4%) and influenza virus (14.4%) were detected as the most common viral agents[16].

It is known that viral respiratory infections are a serious health problem all over the world. These infections cause a heavy burden on the budget in terms of national health expenditures due to the morbidity and mortality they cause. Influenza vaccination has an important place among prevention methods of viral infections. The most common antibiotic prescribed infections are URTIs. Because of inappropriate antibiotic use, URI

has a high cost of treatment. Considering high viral pathogen detection rates by physicians, it may prevent unnecessary use of antibiotics in the treatment of URTI. Detection of these viral agents will reduce the cost of treatment and prevent the development of antibiotic resistance.

CONFLICT of INTEREST

Authors have no competing interests to disclose.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: SM, DF

Analysis/Interpretation: SM, DF

Data Acquisition: SM, DF

Writting: SM, DF

Critical Revision: SM, DF

Final Approval: SM, DF

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