Original Investigation / *Özgün Araştırma*

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Duration of Viral Analysis in Laboratory in the Seasonal Influenza Period

Mevsimsel İnfluenza Döneminde Laboratuvarda Viral Analiz Süresinin Önemi

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Abstract

Objective: Seasonal influenza is an acute viral respiratory tract infection which exhibits annual epidemics worldwide and effects all age groups. Surveillance studies provide monitoring annually circulating viruses. In addition, virus types can be determined in such period targeting patient-based diagnosis. The present study aims to evaluate the effect of duration of the viral classification studies in the laboratory on treatment planning in cases hospitalized due to influenza- like symptoms.

Material and Methods: Children younger than 18 years of age, hospitalized due to symptoms that might be associated with influenza infection between the December 2015 and April 2016 influenza season were tested for Influenza A and Influenza B viruses in nasopharyngeal swab sampling. Demographic characteristics, duration of symptoms, duration to get the viral detection results (polymerase chain reaction: PCR) in the laboratory, and duration of hospitalization were evaluated.

Results: A total of 132 pediatric patients were included in the study and the median age was 27.1 months (IQR (interquartile range): 4-99.7). Fifteen patients (11.3%) were influenza positive by PCR. Empirical oseltamivir treatment was given to 22% of the patients. Among the fifteen patients (11.3%) who were positive for influenza virus, six were administered empirical oseltamivir within the first 48 hours of admission, before the laboratory results were obtained and no antiviral was administered to the others found influenza positive. At the admission, the median symptom duration was 2 days (IQR: 1-4) whereas median hospitalization duration was 7 days (IQR: 4-11). The median duration to get the laboratory results was 8 days (IQR: 6.2-10).

Özet

Giriş: Mevsimsel influenza dünya çapında etkili olan, yıllık epidemiler yaparak her yaş grubundan bireyi etkileyebilen akut viral bir solunum yolu enfeksiyonudur. Sürveyans çalışmaları yıllık olarak dolaşan virüslerin monitörize edilmesi açısından önem taşımaktadır. Bunun yanı sıra bu dönemde hasta bazında tanısal amaçlı viral tiplendirme de yapılabilmektedir. İnfluenza benzeri semptomlarla hastaneye yatırılan olgularda viral tiplendirme için yapılan laboratuvar çalışmalarının sonuçlanma süresinin tedavi planlamasına etkisini araştırmayı amaçladık.

Gereç ve Yöntemler: Hastanemizde Aralık 2015-Nisan 2016 influenza döneminde influenza enfeksiyonuyla ilişkili olabilecek semptomlarla hastaneye yatmış olan 18 yaş altı hastalarda nazofarengeal sürüntü örneklemesi ile influenza A ve B virüsleri için viral tiplendirme yapıldı. Hastaların demografik özellikleri, semptom süreleri, laboratuvardan viral tiplendirme (PCR: Polimeraz zincir reaksiyonu) sonuçlanma süresi ve yatış süreleri değerlendirildi.

Bulgular: Toplam olarak 132 hasta değerlendirmeye alındı, ortanca yaş 27.1 aydı [IQR (interkuartil aralık): 4-99.7]. On beş (%11.3) hastada influenza PCR pozitifliği elde edildi. Çalışma grubumuzda ampirik oseltamivir tedavisi vakaların %22'sine uygulanırken, influenza virüsü tespit edilen 15 hastanın 6'sına ilk 48 saatte laboratuvar sonucu tarafımıza ulaşmadan ampirik oseltamivir başlanmış, diğer influenza pozitifliği saptanan olgularda antiviral uygulanmamıştır. Hastaneye başvuruda ortanca semptom süresi 2 gün (IQR: 1-4), ortanca yatış süresi 7 gündü

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Conclusion: Waiting for the laboratory results may take time in the seasonal influenza infection period for patients with influenza symptoms and findings requiring hospitalization and this is not an effective method in influenza control. Thus, starting the treatment should be preferred at admission, and in the first 48 hours in indicated cases.

Keywords: Seasonal influenza, oseltamivir, influenza test

(IQR: 4-11). Laboratuvardan test sonuçlarının elde edilmesi için geçen ortanca süre 8 gündü (IQR: 6.2-10).

Sonuç: Mevsimsel influenza döneminde hastaneye yatışı gerektiren, influenza semptom ve bulguları ile başvuran hastalarda laboratuvar tetkiklerinin beklenmesi zaman kaybına neden olabilir ve influenza kontrolünde etkin bir yöntem olamaz. Bu nedenle, endike olan durumlarda ilk 48 saat içerisinde olmak üzere başvuru anında tedavi başlanması tercih edilmelidir.

Anahtar Kelimeler: Mevsimsel influenza, oseltamivir, influenza test

Introduction

Influenza is an acute febrile disease, caused by influenza type A and B viruses; it is active every winter in mild climates and all the year around in tropical climates. Its two most important characteristics are that it progresses with epidemics and that it may cause mortality. Regional influenza follow-up studies bear importance for the epidemiology of the disease, risk groups, identification of transmission characteristics and the assessment of the effect of the disease. Many influenza cases, without underlying diseases and with typical symptoms, require specific viral diagnosis confirmation in the epidemic stage (1). However, diagnostics tests may be applied if the following clinician thinks that they will affect the clinical approach. The clinical approach includes antiviral and antibacterial treatments and the infection control measures (1). The Centers for Disease Control and Prevention (CDC) recommends that empirical oseltamivir treatment is started as soon as possible for all patients hospitalized with influenza infection suspicion, without waiting for the influenza test results (2).

In the light of these information, we aimed to investigate the effect of the duration of laboratory studies for viral characterization in cases hospitalized with influenza-like symptoms in the epidemics season.

Materials and Methods

The present study prospectively includes pediatric cases between 0-18 years old, admitted to the Pediatric Hospital with influenza-like symptoms in the influenza season between December 2015-April 2016 and hospitalized, treated and monitored. The present study has been approved by the Non-Interventional Clinical Research Ethical Board. The cases hospitalized with influenza-like symptoms (body temperature 38°C or higher cough and/or sore throat) were included in the study. The influenza virus identifications in the nasal and nasopharyngeal swab samples taken from the mentioned cases within 48 hours after admission to the hospital were conducted with the multiplex real-time polymerase chain reaction (RT-PCR) using the multiplex Influenza A,B,C kit (TIBMOLBIOL, Germany) and a Bio-Rad CFX 96 platform instrument (Bio-Rad, USA). In positive samples, Influenza A H1N1, Influenza A H3N2 and Influenza B characterization was conducted usin the same instrument and CDC primers and probes (3). The results were sent to us by the laboratory the same day they were concluded.

The cases were assessed with respect to age, gender, the underlying disease, symptom duration at referral to the hospital, whether oseltamivir treatment was administered within 48 hours upon hospitalization, total hospitalization duration, whether virus has been identified with RT-PCR and the virus subtypes, the time that has taken the results made available to us and mortality associated with influenza.

The data were analyzed using the IBM SPSS Statistics (Windows, Version 22.0. Armonk, NY: IBM Corp.) software. In the evaluation of basal characteristics of the cases, descriptive statistics was used for average, median, standard deviation, and interquartile range for the numerical variables and frequency distributions for categorical variables.

Results

The present study included 132 cases [64 females (48.5%), 68 males (51.5%)]. The median age was 27.1 months (IQR:4-99.7) (Table 1). 49% of the cases were under 2 years old (under 6 months 30%, 6 months -2 years old 19%) (Figure 1). In 43% (n= 57) of the patients, there was no underlying disease. In the cases with an underlying disease, the most frequent ones were neurometabolic disease (n= 24), chronic lung disease (n= 13) and prematurity (n= 11).

It was observed that in 22% (n= 29) of the cases after hospital admittance empirical oseltamivir treatment was started. There was no influenza associated mortality. 9 of the cases (6.8%) were under observation in the intensive care unit. When the PCR results were assessed, the influenza virus was detected in 11.3% (n= 15) of the cases. Influenza A H1N1 was detected in 7 cases; Influenza A H3N2 was detected in 4 cases; İnfluenza B was detected in 2 cases. In two cases, an Influenza A virus other than Influenza A H1N1 and H3N2, subtypes were

Table 1. Characteristics and the results of the cases

Age* (months) (IQR)	27.1 (4-99.7)
Gender**(%)	
Female	64 (48.5)
Male	68 (51.5)
Underlying disease**(%)	
No	57 (43)
Yes	75 (57)
Neurometabolic disease	24
Chronic lung disease	13
Prematurity	11
Hemato-oncological malignity	7
Congenital cardiac disease	7
Primary immunodeficiency	6
Chronic renal disease	2
Gastrointestinal disease	3
Rheumatologic disease	1
Hematologic disease	1
PCR result**	
Influenza (+)	15 (11.3)
Influenza A H1N1	7
Influenza A H3N2	4
Influenza A (H1 ve H3 dışı)	2
Influenza B	2
Influenza (-)	117 (88.6)
Empirical antiviral treatment**(%)	
No	29 (22)
Hayır	103 (78)
Influenza associated mortality	0

* The values are given in median and interquantile range (IQR).
** The values are given in numbers and percentages.
PCR: Polymerase chain reaction.

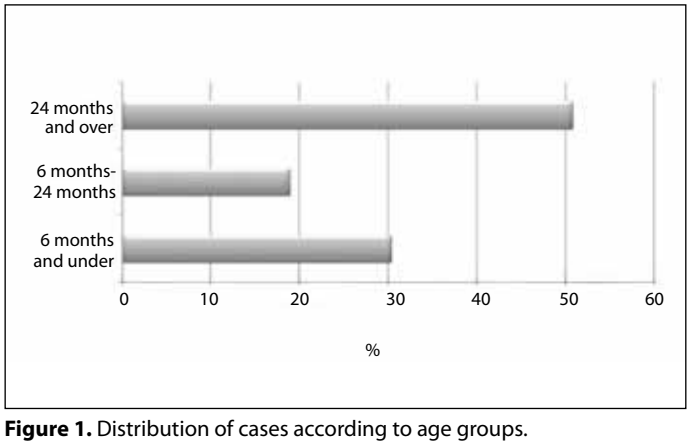

Figure 1. Distribution of cases according to age groups.

Table 2. Time evaluation of the cases

Symptom duration*(day) (IQR)	2.0 (1-4)
Hospitalization duration*(day) (IQR)	7 (4-11.7)
PCR result duration*(day) (IQR)	8 (6.25-10)

* The values are given in median and interquantile range (IQR).

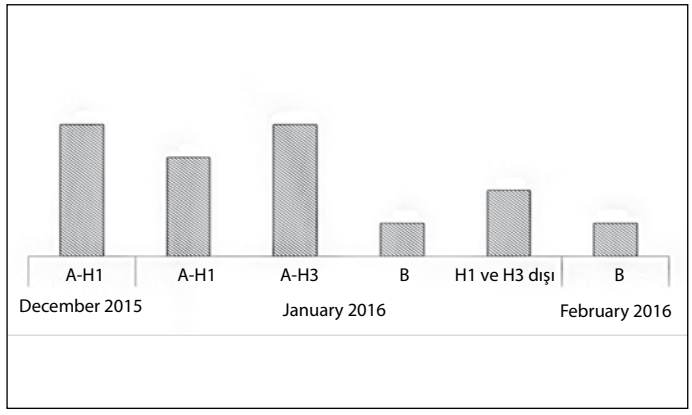

Figure 2. Distribution of influenza infection detected cases according to time and virus type.

not characterized (Table 1). Empirical oseltamivir treatment was administered to 40% (n= 6) of the cases where influenza infection was detected. The distribution of the cases according to months is given in Figure 2. Of the cases where influenza infection was detected, 7 (47%) were under 2 years old.

For all the cases, median symptom duration at admittance was 2 days (IQR: 1-4), median hospitalization duration was 7 days (IQR: 4-11.7), the median duration between admittance and the result of the PCR from the laboratory being available was 8 days (IQR: 6.25-10) (Table 2).

Discussion

In the present study, it was observed that the laboratory results for children hospitalized due to influenza-like symptoms in the 2015-2016 influenza season could not be available rapidly so as to guide the planning of the treatment. The laboratory work for routine virus detection and characterization, except for the surveillance studies in the influenza season, causes time loss and it has been shown that this is not an effective method in influenza control. Thus, the recommended approach should be central monitoring and starting direct treatment for selected patients upon the detection of the infection. Although the times it takes to get the influenza test results varies depending on the working conditions of the laboratories, virus identification, isolation and characterization may take time. In addition, increase of the number of cases in the influenza season may be one of the factors that affect the speed of the laboratory to give the results.

Clinical assessment for pediatric patients with influenza-like diseases, based on factors such as the underlying disease, the severity of the disease, time from the start of the symptoms and the regional influenza activity is the most important factor for the decision to start antiviral treatment (2). It is recommended that antiviral treatment in hospitalized, high risk patients is started in the shortest time possible,

without waiting for the results of the influenza test since early start of treatment will give the best results. Diagnostic influenza tests vary with respect to method, sensitivity and cost. The American Pediatric Academy recommends the conductance of influenza tests if the results will be obtained rapidly so as to provide clinical assessment and taking infection control measures (4).

In population-based, laboratory confirmed seasonal influenza studies, it is reported that influenza associated hospitalization is most common in children under 2 years old and that the highest risk group is the 6 months old children (5-7). Similarly, in surveillance studies conducted to assess disease load, it has been observed that influenza-associated complications are more frequent especially in children under 2 years old. Quach et al. report that 34% of the pediatric patients hospitalized due to influenza-associated infection are under 6 months old (8). In parallel with the literature, in the present study, 49% of the screened cases were under 2 years old and 30% were in the under 6 months age group. In addition, there was an underlying disease in 57% of the cases and the most frequent disease groups were neurometabolic diseases and chronic lung disease. The most risky groups with respect to influenza complications were stated by the CDC; and those with chronic lung disease, cardiac disease, metabolic diseases such as diabetes mellitus, hereditary metabolic diseases, hematologic diseases and neurodevelopment problems as underlying diseases were found to be the most frequent (9,10).

The virus spreads for 5 to 10 days (11). In young children high titer of virus removal may take longer time since immunity is yet inadequate (12). In the present study influenza virus was positive by PCR in 11.3% of the cases. Subtype characterization identified influenza A H1N1 as the most frequent. The CDC, in its paper on influenza surveillance in the United States of America (USA) in the 2015-2016 season, reported that of the influenza viruses in general in the USA; 70.8% was Influenza A and 29.2% was Influenza B; subtype results were 80.7% H1N1 and 19.3% H3N2 for Influenza A and 68.5% B/Yamagata and 31.5% B/Victoria for Influenzas B (13). According to the 2015-2016 Sentinel Influenza-like Disease Surveillance, conducted by the Turkish Public Health Directorate, the disease activity started in the 50th week of 2015 and reached maximum level in the 2nd week of 2016. In this period, influenza positivity reached 60%, and the most frequent one was detected to be influenza A H3N2 (14). The differences in the results of the patient group screened in the present study may be due to the fact that the patient group is a pediatric age group, that risky cases are more frequently admitted to our hospital which is a third step diagnosis and treatment center, and that the present study covers only the hospitalized patients.

As a consequence of observational studies assessing the effectiveness of oseltamivir in all age groups, oseltamivir shows the highest effect when started within the first 48 hours following the onset of the symptoms; however, it has been reported that there will be significant reduction in mortality and critical morbidity when stared within 5 days (15-17). The CDC recommends early antiviral treatment since the age group younger than 2 years old is highly risky with respect to complications, and oseltamivir treatment for patients smaller than 1 year old is effective and may be used (2,18). In our study group, empirical oseltamivir treatment was administered to 22% of the cases; of the 15 influenza virus detected patients, empirical oseltamivir treatment was started for 6 patients in the first 48 hours, before the laboratory results were available to us and antiviral was not applied for the other influenza positive cases. In addition, 5 of the 6 cases for which empirical oseltamivir treatment was started were older than 24 months. These resultrs show that our empirical oseltamivir treatment applications are behind the recommended with respect to both age and frequency.

In conclusion, waiting for the results of the laboratory assays will cause loosing time and missing the most effective phase of the antiviral treatment especially in risky groups requiring hospitalization in the influenza season. Thus, waiting for the results of the laboratory assays can not be an effective method in disease control and prevention of complications in the influenza season. It is important that the empirical antiviral treatment is started within the first 48 hours especially in indicated cases under 2 years old.

Ethics Committe Approval: The study was approved by Hacettepe University Clinical Research Ethics Committee with decision numbered GO 15/766.

Informed Consent: Informed was received from the patients and parents.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – AK; Design – STB; Data Collection and Processing - OG, STB, MT; Analysis and Interpretation - AK, STB, KA, ÖT; Writing – STB; Confirmation - ÖT, AK.

Conflict of Interest: No conflict of interest was declared by the authors.

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