Clinical Analysis in Influenza A (H1N1) Virus Patients

İnfluenza A (H1N1) Virus Enfeksiyonlu Hastaların Klinik Analizi

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ABSTRACT

Objective: The first influenza virus pandemic of the 21st century began in Mexico in 2009 and spread rapidly all over the world. We described the clinical and epidemiologic characteristics of the 31 patients admitted to the Deparment of Chest Disease due to influenza A (H1N1) virus infection.

Material and Method: In this study we evaluated 51 patients with high clinical suspicion for Influenza A (H1N1) virus infection. We used real time reverse transcriptase chain reaction test (RT-PCR) test for diagnosis. Thirty-one (15 female, 16 male) of 51 patients RT-PCR test were positive for Influenza A (H1N1) virus infection. Demographic features, clinical and laboratory characteristics of these patients were assessed.

Results: The mean age of Influenza A (H1N1) virus infected patients was 40.8±13.1. Cough was the most common symptom (90%) and 11 patients (35%) had pneumonia. Leucopenia (48.3%), C reactive protein elevation (90.3%) and creatine phosphokinase elevation (71%) were notable laboratory findings. T and B lymphocyte subgroups were also evaluated and compared with Turkish adult normals. Lymphocyte subgroups were not different in the patients with influenza A (H1N1) virus statistically.

Conclusion: Influenza virus may cause pandemias with different subgroups. Although most Influenza A (H1N1) virus infected patients hospitalized in our clinic had progressed well but pulmonary complications should be considered.
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Key words: H1N1 Influenza A (H1N1) virus infection, symptoms, prognosis

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ÖZET

Amaç: Yirmibirinci yüzyılın ilk influenza virüs pandemisi Meksika'da başladı ve hızla tüm dünyaya yayıldı. Biz bu çalışmada, göğüs hastalıkları kliniğimizde; influenza A (H1N1) virüsü infeksiyonu ile takip edilen 31 hastanın klinik ve epidemiyolojik özelliklerini tanımlamayı amaçladık.

Gereç ve Yöntem: Çalışmaya influenza A (H1N1) virus enfeksiyonu için yüksek klinik şüphesi olan 51 (15-55 yaş) hasta alındı. Influenza A (H1N1) virus enfeksiyonu tanısında gerçek zamanlı revers transkriptaz zincir reaksiyonu (RT-PCR) testi kullanıldı. Çalışmaya alınan 51 hastanın 31'inde (15 kadın, 16 erkek) influenza A (H1N1) virus için RT-PCR testi pozitif bulundu. Bu hastaların demografik verileri, klinik ve laboratuvar özellikleri, tedaviye cevapları değerlendirildi.

Bulgular: Influenza A (H1N1) virus enfeksiyonlu hastaların yaş ortalaması 40.8±13.1 idi. En sık semptom öksürük (%90) iken, 11(%35) hastada pnömoni geliştiği görüldü. Lökopeni (%48.3), C reaktif protein (%90.3) ve kreatinin fosfokinaz (%71) yüksekliği belirgin laboratuvar bulgular idi. Ayrıca serumda T, B lenfosit alt grupları akım-sitometrik olarak incelenerek normal Türk erişkin değerleri ile karşılaştırıldı. Influenza A (H1N1) virus enfeksiyonun lenfosit alt gruplarında istatistiksel olarak anlamlı bir farklılık yapmadığı görüldü.

Sonuç: İnfluenza virus değişik alt tipleri ile pandemilere yol açabilen bir virüstür. Kliniğimizde takip edilen influenza A (H1N1) virus enfeksiyonlu hastaların prognozlarının kötüye gitmemesi yüz güldürücüdür ancak yine de pulmoner komplikasyon gibi sistemik etkileri gözardı edilmemelidir.
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Anahtar sözcükler: Influenza A (H1N1) virüs enfeksiyonu, semptomlar, prognoz

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INTRODUCTION

Until recently, H5N1 avian strains of influenza were the predominant focus for surveillance of potential new pandemic viral strains. Novel influenza A (H1N1) virus is the pathogen of recent global outbreaks of febrile respiratory infection. Influenza viruses that infect humans are classified into 3 principal types: A, B, and C. While B and C are usually human-specific, influenza A virus is preferentially endemic in water birds, which usually do not fall ill with this infection [1]. However, some subtypes have adapted to other birds (chickens) and mammalians (pig, horse, humans) in species-specific strains [2]. In humans, three types of influenza A virus (H1N1, H2N2, H3N2) verifiably caused pandemics of high morbidity and mortality [3]. The novel H1N1 influenza virus represents

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quadruple types of 1 human, 1 avian, and 2 swine strains (North American and Eurasian) of influenza virus [4]. Hemagglutinin (HA) and neuraminidase (NA) are the important antigenic proteins on the surface of the virus and both undergo two types of antigenic variation, drift and shift. Antigenic drift involves minor changes in these antigens, while shift involves major changes in these molecules that result from replacement of a gene segment(s). New viral variants, due to antigenic drift, emerge constantly and are responsible for yearly epidemics. In contrast, antigenic shifts can produce new virus strains to which most people have no immunity resulting in pandemics [5]. Three pathogenic viruses, the 1918 Spanish flu (H1N1), 2003 avian flu (H5N1) and recently the 2009 swine flu (A/H1N1), share the same subtype 1 (N1) of neuraminidase which is a glycoprotein component on a flu virus' surface. The symptoms of this H1N1 influenza in people include fever, cough, sore throat, body aches, headache, chills and fatigue. A probable case with influenza A (H1N1) virus is defined as a person with high fever (≥38°C) and/or at least two acute respiratory symptoms along with epidemiological criteria listed in the case definition protocol published by WHO (World Health Organization) [6]. However, the 2009 outbreak has shown an increased percentage of patients reporting diarrhea and vomiting [7].

MATERIALS and METHODS

Patients

In this study, we evaluated 51 patients (24 female, 27 male) who were hospitalized with suspection of influenza A (H1N1) virus at the Chest Diseases Clinic in Ankara Dışkapı Yıldırım Beyazit Research Hospital, in the last three months of 2009. A total of 51 patients were screened; 31 were enrolled in the study. There was a high clinical suspicion in 51 patients for influenza A (H1N1) virus infection. RT-PCR test was positive in 31 patients. Sixteen of them were men and fifteen were women. Their mean±SD age was (40.8±13.1). No underlying medical history was reported in any patient.

Hospitalized cases were selected with at least two acute respiratory symptoms along with epidemiological criteria listed in the H1N1 case definition protocol published by WHO. A case with H1N1 virus was defined as a person with high fever (≥38°C) and/or also have symptoms like cough, sputum, headache, nausea, vomiting and rhinorrhea. All patients were given Oseltamivir (75 mg/day). One of the patients developed acute respiratory failure.This patient were included in the intensive care unit and were given non-invasive mechanical ventilation, Oxygen therapy. The patients' general condition improved after 4 days.

Enrolled patients underwent a detailed work-out comprising case history and physical examination, chest X- ray, thorax tomography if considered necessary, white blood cell count (WBC), C reactive protein (CRP), aminotransferase (ALT and AST), Lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) levels.

Samples

The symptoms of patients for influenza A (H1N1) virus started 1-2 days before hospital admission. Nasal swabs and blood samples were obtained from hospitalized patients (in the first two day) by their physicians, following the procedures and appropriate consent protocols approved by the Human Subjects Committee of the hospitals. Nasal and/or nasopharyngeal samples taken from suspected cases and transported in a viral transport medium (Virocult, Medical Wire&Equipment, UK) to Refik Saydam National Public Health Agency (RSNPHA) National Influenza Center (Ankara, Turkey) along with patient information forms. The samples coming in transport medium were gently mixed by vortex and the samples were transferred into microcentrifuge tubes in a Biological Safety Cabinet. For detection of influenza A (H1N1) virus, RNA extraction was done with EZ1 virus Mini Kit and Qiasymphony Virus/Bacteria Mini Kit according to the kit instructions in EZ1 Advanced XL and Qiasymphony isolation machine *(Qiagen Gmbh, Hilden, Germany)*. Influenza A(H1N1) virus PCR performed with Qiagen artus Infl/H1 LC/RC RT-PCR commercial kit which were directly searching for a 80 base pair region of pandemic influenza A (H1N1) virus by using the Rotor-Gene 6000 *(Germany)* PCR instrument.

Lymphocyte subgroups analysis of all patients were made by flowcytometric analysis (FACSCalibur, Becton-Dickinson (BD), Erembodegem, Belgium), from peripheral blood samples. BD Multitest kit, CD3 FITC/CD16 + CD56 PE/CD45 PerCP/CD19 APC and CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC was used for lymphocyte subgroups analysis. The BD Multitest™ kit is a four-color direct immunofluorescence reagent kit for use with flow cytometer to identify and determine the percentages of human lymphocyte subsets in erythrocyte-lysed whole blood. Lymphocyte subgroups reference value of BD Multitest kit was used to evaluate the results.

In addition, WBC, CRP, ALT, AST, LDH and CPK serum levels were also analyzed for all patients.

Twenty patients presented with flu-like syndrome; their mean±age was 41.6±15.2 years; 12 were male and eight female. Fever and malaise were the predominant symptoms occurring in all patients (100%) followed by cough in six (43.6%) patients. Twenty patients did not have a influenza A (H1N1) virus infection. Ten of them had acute bronchitis, six of them had upper respiratory tract infection, four had communutiy acquired pneumonia. No underlying medical history was reported in any patient. None of these patients developed pneumonia. Infection convalescence was noted in all.

Statistical Analysis

Data were analyzed with SPSS for Windows 11.5 pocket programme. Nominal variables were analyzed with Chi square test. Whether the distributions of continuous data were normal or not was evaluated by Shapiro Wilk's test. Student's t test was used to

investigate the association of H1N1 infection with variables like age, CD3, CD4 and NK cells (i.e normally distributed data) and Mann-Whitney U test was used for the association with CD8, CD4/CD8 and B cell levels (i.e not normally distributed data). Results are accepted as statistically significant for $p<0.05$.

Ethics Statement

The study was performed in the last three months of 2009. The study protocol was approved by the Ethics Committee of the Dışkapı Yıldırım Beyazit Research and Education Hospital of Ankara. All patients admitted to the chest disease department for flu-like symptoms and who provided written-informed consent were eligible.

RESULTS

H1N1 influenza virus infection was confirmed with real time RT-PCR in 15 female and 16 male patients. Their mean±SD age was 40.8±13.

Clinical symptoms and laboratory findings of group

The most common presenting symptoms of patients were cough, fever and sore throat. In H1N1 influenza positive patients; cough (90%), sore throat (64.5%), fever of >38°C (64.5%), coryza (52%), headache (48%), myalgia (32%) and weakness (29%), diarrhea (19%) and vomiting (16%) were reported, respectively. Eleven patients from the H1N1 positive group developed H1N1 influenza-related pneumonia.

The liver enzymes (AST, ALT) were slightly elevated in our group without underlying liver disease. The level of plasma CRP was elevated 90% in the patients of H1N1 influenza. The level of CPK was elevated 71% in the patients of H1N1 positive influenza (Table 1).

Lymphocyte subgroup analysis

H1N1 positive patients, the percentage of T cell (CD3+), T helper cells (CD3+CD4+), cytotoxic T cell (CD3+CD8+), natural killer (NK) (CD3-/CD16+56+) cell, and B cell (CD19+) were all normal compared to normal values for Turkish adults.

H1N1 influenza virus infected patients

Mean rates of CD3+, CD3+CD4+, and CD3+CD8+ lymphocytes in H1N1 influenza virus positive patients were 68.9±9.2%, 42.5±7.0% and 29.8±7.4%, respectively. Mean rates of B cell and NK cells in H1N1 influenza virus positive patients were 15±04% and 14.5±7.3%, respectively.

There was no statistically significant ($p>0.05$) difference between Influenza A(H1N1) pneumonia (+) and pneumonia (-) patients in T cell, B cell and NK cell levels (Table 2, Figure 1).

DISCUSSION

Since pandemic Influenza A (H1N1) virus 2009 was first identified in Spring 2009, there has been a worldwide dissemination of influenza virus. In the last three months of 2009, a total of 31 patients were hospitalized with suspicion of flu [influenza A (H1N1) virus] and screened for H1N1 influenza. Influenza A (H1N1) virus infection was confirmed in 31 patients with real time RT-PCR.

Recent reports indicating that age-specific attack rates for the 2009 pandemic influenza A (H1N1) virus infection are higher in younger persons and lower in older person [8].The ages of our patients with influenza A (H1N1) virus were between 15-55 yrs. Their mean± SD age was 40.8±13. Among the 31 patients, 16 and 15 were males and females, respectively. 9.7% were between 15-20 years old, 20 were between 20-45 years old (64.5%) and 8 were between 45-55 years old (25.8%).

Pneumonia is recognized as the most important complication of influenza infections [4, 9]. In our study, 35% of influenza A (H1N1) virus groups had pneumonia.

Table 1. Characteristics of 31 patients with influenza A (H1N1) virus

Variables	n	(%)
Sex		
Female	15	48.4
Male	16	51.6
Age (years)	Mean±SD (range) 40.8±13 (15 to 55)	
15 to 20 years	3	9.7
20 to 45 years	20	64.5
45 to 55 years	8	25.8
Symptoms		
Cough	28	90.3
Sore throat	20	64.5
Fever (≥38°C)	20	64.5
Coryza	16	51.6
Headache	15	48.4
Myalgia	10	32.3
Weakness	9	29.0
Pneumonia	11	35
Laboratory		
Leukopenia (white-cell count <4000/mm^3	15	48.3
Leucocytosis	9	29
(white-cell count >10.000/mm^3		
Increased CPK (0-180 U/L)	22	71.0
Increased AST (0-40 U/L)	9	29.0
Increased ALT (0-41 U/L)	6	19.4
Increased CRP (0-5.0 mg/L)	28	90.3
Increased LDH (207-414 U/L)	8	25.8

SD: Standard deviation, CPK: Creatine phosphokinase, AST: Alanine amiotransferase, AST: Aspartate aminotransferase, CRP: C reaktive protein, LDH: Lactate dehydrogenase

Table 2. The percentage of lymphocytes in H1N1 (+) patients with pneumonia (+) and pneumonia (-)

	H1N1 pneumonia (+)	H1N1 pneumonia (-)	p
T CellCD3(+)	63.9±13.93	69.2±8.24	0.267†
T CellCD4(+)	40.5±8.35	43.7±6.25	0.242†
T CellCD8(+)	28.5±9.03	31.4±8.14	0.289‡
CD4/CD8	1.4±0.49	1.5±0.38	0.785‡
B Cell (CD19+)	19.9±11.04	15.9±7.44	0.411‡
NK Cell (CD3-CD16+CD56-)	12.3±5.97	16.4±7.84	0.139†

†Student's t test, ‡Mann-Whitney U test

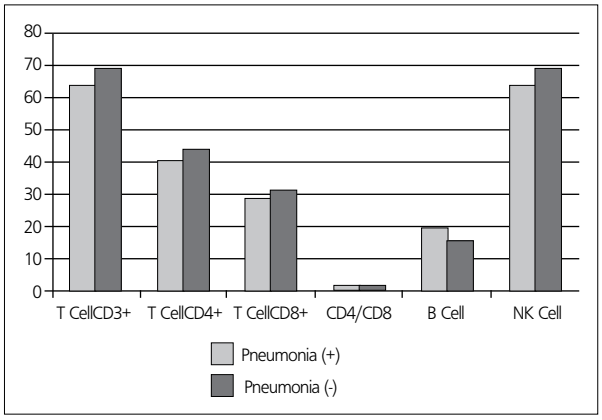

Figure 1. Difference between Influenza A (H1N1) pneumonia (+) and pneumonia (-) patients i immune system cells

There was no statistically significant difference between Influenza A (H1N1) pneumonia (+) and pneumonia (-) patients in immune system cells.

A striking feature observed in this cohort of patients was the high frequency of systemic involvement with laboratory evidence of myositis/rhabdomyolysis, elevated acute-phase reactants. Myositis (with or without rhabdomyolysis) has previously been associated with influenza infections [10]. Elevation of LDH, CPK levels has been reported for patients with seasonal influenza but may not be considered a specific marker of severity. The CPK levels were higher in our patients. In our cohort, 48.3% of 31 influenza A (H1N1) virus patients had leukopenia (white-cell count <4000/mm^3). Leucocytosis was reported in 29% of influenza A (H1N1) virus patients. Both, leucocytosis and leukopenia were reported in hospitalized patients with influenza A (H1N1) virus in another study [11]. In our series, lymphopenia and thrombocytopenia did not develop but they were common findings in all series in avian influenza virus infection; these were prognostic indicators of ARDS and death [12,13].

The great majority of influenza A (H1N1) virus infections are mild and self limiting in nature and influenza A (H1N1) virus pandemic appears to have a high infectivity rate but low pathogenicity. However, recent studies have shown that the high fatality rate of avian influenza H5N1 virus infections is a consequence of an overactive inflammatory response and the severity of infection is closely related with virus-induced cytokine dysregulation. The privileged cells of cytokine storm are macrophages and CD8(+) T-lymphocytes, while the primary contributor cytokines are TNF-alpha, IL-6 and IFN-gamma [14]. Influenza A (H1N1) virus infection remains relatively milder than H5N1 infection, although a small minority of patients appears to manifest with a primary viral pneumonia that may progress to an ALI or ARDS-like clinical presentation.

Very limited evidence has been reported to show human adaptive immune responses to the influenza A (H1N1) virus. In a study, [15] findings suggest that without protective antibody responses, individuals vaccinated against seasonal influenza A may still benefit from preexisting cross-reactive memory CD4(+) T cells reducing their susceptibility to influenza A (H1N1) virus infection. Previous exposure to seasonal H1N1 viruses, either through natural infection or through vaccination, presumably led to the generation of these memory T cells.

Several previous reports showed that the presence of memory T-cell responses against H5N1 avian flu virus in healthy individuals resulted from seasonal flu vaccination [16,17]. A recent publication showed that a prior exposure to H1N1 and H3N2 seasonal influenza A virus strains provided partial immunity against influenza A (H1N1) virus infection in guinea pigs [18].

In our small group, influenza A (H1N1) virus infection did not show a major effect on the clinical and laboratory values. Although the rapid spread of the virus increases the number of cases, mortality rate still is low and many patients do not need hospitalization. So, this situation may indicate that influenza A (H1N1) virus infection is a relatively mild viral infection and is not expected to cause serious clinical symptoms with changing the cellular pattern of the immune system.

Conflict of Interest

No conflict of interest was declared by the authors.

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