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ARAŞTIRMA ● RESEARCH ARTICLE

TNF-α, IL-1β and IL-6 Levels in Pandemic Influenza A (H1N1) 2009 Patients and Effect on Mortality

Pandemik İnfluenza A (H1N1) 2009 Hastalarında TNF-α, IL-1β, IL-6 Düzeyleri ve Mortaliteye Etkisi

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ABSTRACT

Introduction: Throughout history, influenza virus pandemics have led to the death of millions of people. The virus sometimes causes pathological changes that can lead to severe illness and death. Inflammatory cytokines and chemokines have been shown to be involved in the pathogenesis of tissue damage in the lungs of animals and humans infected with influenza viruses.

Materials and Methods: The serum concentrations of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) were determined with enzyme immunoassay (EIA) in 57 patients who were hospitalized with confirmed influenza and a control group.

Results: Fifty-seven patients with confirmed influenza A (H1N1) 2009 and 62 healthy subjects as the control group were included in this study. Of these patients with influenza, 51 (89.4%) were discharged, and 6 (10.5%) died of influenza-related illness. TNF-α levels were found to be 43.0 pg/mL in fatal patients, 20.9 pg/mL in non-fatal patients, and 4.1 pg/mL in the control group. IL-6 levels were found to be 1074.12 pg/mL in fatal patients, 191.0 pg/mL in non-fatal patients, and 36.1 pg/mL in the control group. The differences between groups were statistically significant ($p= 0.003$ and $p< 0.001$, respectively). IL-1β levels were found to be 2.1 pg/mL in fatal patients, 7.1 pg/mL in non-fatal patients, and 7.5 pg/mL in the control group, and the difference was not statistically significant ($p= 0.657$).

Conclusion: We found that TNF-α and IL-6 levels were significantly higher in patients who died. We suggest that higher levels of pro-inflammatory cytokines may be used as an important marker of mortality.

Key words: Influenza A virus H1N1 subtype, tumor necrosis factor-alpha, interleukin-1beta, interleukin-6.

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ÖZET

Giriş: İnfluenza virüs tarih boyunca birçok pandemiler yaparak milyonlarca insanın ölümüne yol açan önemli bir hastalık etkenidir. Akciğerde patolojik değişikliklere neden olarak ağır hastalık ve ölüme neden olabilir. Akciğerlerde oluşan hücresel infiltrat ve doku hasarında doğal immünitenin bir parçası olan sitokinlerin rolü bilinmektedir. İnfluenza virüsün bazı hastalarda oluşturduğu aşırı ve kontrolsüz sitokin salınımının (sitokin fırtınası), hastalığın ağır seyrinden sorumlu olabileceği düşünülmektedir.

Materyal ve Metod: Hastaneye pandemik influenza ön tanısıyla yatırılan ve referans laboratuvarında RT-PCR ile teyit edilmiş influenza A (H1N1) tanısı konan hastalar ve tamamen sağlıklı kontrol grubunun serumlarında, enzim immünoassay (EIA) yöntemiyle tümör nekroz faktörü-alfa (TNF-α), interlökin 1-beta (IL-1β) ve interlökin 6 (IL-6) çalışıldı.

Bulgular: Çalışmaya 57 konfirme H1N1 hastası, 62 kontrol grubu sağlıklı kişi dahil edildi. Hastaların 6 (%10.5)'sı takipleri sonunda hayatını kaybederken, 51 (%89.4) hasta şifa ile taburcu edildi. TNF-α ölen hastalarda 43.0 pg/mL, yaşayan hastalarda 20.9 pg/mL, kontrol grubunda 4.1 pg/mL ölçülürken, IL-6 değerleri ölen hastalarda 1074.1 pg/mL, yaşayan hastalarda 191.0 pg/mL, kontrol grubunda 36.1 pg/mL ölçüldü ve aradaki fark istatistiksel olarak anlamlıydı (sırasıyla; p= 0.003 ve p< 0.001). IL-1β değerleri ise ölen hastalarda 2.1 pg/mL, yaşayan hastalarda 7.1 pg/mL iken, kontrol grubunda 7.5 pg/mL ölçüldü ve aradaki fark istatistiksel olarak anlamlı değildi (p= 0.657).

Sonuç: TNF-α ve IL-6 düzeylerinin ölen hastalarda belirgin yüksek saptanması fatalite belirteci olarak kullanılabileceğini düşündürmektedir.

Anahtar kelimeler: İnfluenza A virüsü H1N1 alttip, tümör nekroz faktörü-alfa, interlökin-1 beta, interlökin-6

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INTRODUCTION

Influenza is an important viral disease that spreads through the respiratory tract and causes pandemics. Although it generally leads to an illness with signs and symptoms like fever, headache, muscle pain, malaise, and cough, and resolves spontaneously, it sometimes causes pathological changes that can lead to severe illness and death. It is known that seasonal influenza pandemics affect about 3-5 million people every year and cause the death of 250.000-300.000 people annually[1]. Antigenic drift in influenza A may result in new viruses and may give rise to new pandemics due to lack of immunity in the population. This can cause infection and the death of many more people[2].

In the fight against influenza, vaccines and protective measures are used to prevent transmission of the disease, and antiviral drugs are used for treatment. In addition to antiviral drugs, immunosuppressive drugs have been used to halt excessive and uncontrolled cytokine secretion (cytokine storm) that influenza virus causes in animal models[3].

The aim of this study was to determine the effects of proinflammatory cytokines on mortality in patient with influenza A (H1N1) 2009 infection.

MATERIALS and METHODS

Patients

Among the patients who received inpatient treatment at Diskapi Yildirim Beyazit Training and Research Hospital with the preliminary diagnosis of pandemic influenza A (H1N1) 2009 infection, those with laboratory-confirmed influenza were included in this study. The hospitalization criteria for the patients with preliminary diagnosis of pandemic influenza included body temperature ≥ 38.3°C and one of the following symptoms: shortness of breath and respiratory distress, oxygen saturation ≤ 92% measured by pulse oximeter, and changes in vital signs (arterial hypotension, increase in respiration rate, increase in heart rate, impaired consciousness, severe dehydration, abnormal chest radiography, or fever ongoing more than three days in spite of treatment with analgesics). High fever was not a requisite in patients older than 65 years, immunosuppressed patients and primary immunocompromised patients. The laboratory diagnoses of the patients were confirmed by the influenza reference laboratory in Refik Saydam Hifzissihha Institute, Ankara. The nasopharyngeal and nasal samples taken within two hours after hospitalization were examined for influenza A virus (H1N1) 2009 by real-time-polymerase chain reaction (RT-PCR).

All patients with influenza A virus (H1N1) 2009 infection received oseltamivir on admission. The standard dose (150 mg/day) was administered for patients with mild disease, and a higher dose (300 mg/day) was used for critical patients. Empirical antibiotic therapy was given to all patients with pneumonia, and secondary bacterial pneumonia was not diagnosed in any patient. The study protocol was approved by the Ethics Committee for Clinical Research of Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey.

Control Group

Sixty-two blood donors with normal physical examination and with no known disease were included in the study.

Cytokine Assay

The blood samples of the patients hospitalized with preliminary diagnosis of pandemic influenza were taken within two hours after admission. After coagulation of the samples, they were centrifuged, and sera were separated immediately and stored at -70°C until use. Cytokine levels were measured with commercially available enzyme immunoassay kits (EIA) (tumor necrosis factor (TNF)-α-EASIA KAP1751, interleukin (IL)-1β-EASIA KAP1211, IL-6-EASIA-CE KAP1261, Diasource, Belgium). With zero attachment, and within 2 standard deviations of average OD values, the lower limits of detection of the tests were as follows: TNF-α: 0.7 pg/mL, IL-1β: 0.35 pg/mL and IL-6: 2 pg/mL.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 15.0 package program was used for statistical analysis. ANOVA, chi-square and Mann-Whitney U tests were used for comparisons. A value of $p< 0.05$ was accepted as statistically significant.

RESULTS

Fifty-seven patients (29 males, 28 females) and 62 healthy controls (31 males, 31 females) were included in the study. The average age of the patients was 44.7 (SD= 17.8; min= 18, max= 83) and of the control group was 42.3 (SD= 9.1). No significant difference existed between the two groups according to gender and age ($p= 0.924$, $p= 0.750$, respectively). Six of the patients (10.5%) died during follow-up, and 51 patients (89.4%) were discharged with full recovery. Demographic characteristics and underlying comorbidities of the patients are shown in Table 1 and the clinical characteristics of the patients are shown in Table 2.

Measured TNF-α, IL-1β and IL-6 levels of the patients and control group are given in Table 3. Levels of TNF-α and IL-6 were higher in H1N1 patients than in the control group and were higher in patients who died during follow-up than in surviving patients, and the difference between them was statistically significant (Table 3, Figures 1,2). While IL-1β levels were higher in the control group than in H1N1 patients, they were at the lowest level in exitus patients, but the difference was not statistically significant (Table 3, Figure 3).

Table 1. Demographic characteristics and underlying comorbidities

Demographic Characteristics	
Sex, n (%)	
Male	29 (50.8)
Female	28 (49.3)
Age (years ± SD)	44.7 ± 17.3
Age group, n (%)	
18-40	22 (38.6)
41-65	26 (45.6)
> 65	9 (15.8)
Duration of symptoms before admission, median days	4.2 ± 2.9
Comorbidities, n (%)	
Diabetes mellitus	12 (21.1)
Chronic obstructive pulmonary disease	9 (15.8)
Asthma	9 (15.8)

Table 2. Clinical characteristics of the patients

Symptoms	**n (%)**
Fatigue	66 (89.2)
Cough	68 (91.9)
Sore throat	27 (36.5)
Myalgia	50 (67.6)
Headache	34 (45.9)
Nasal discharge	15 (20.3)
Shortness of breath	47 (63.5)
Nausea and/or vomiting	25 (33.8)
Chest pain	12 (16.2)
Diarrhea	6 (8.1)
Physical Examination	**n (%)**
Temperature	
36-37.9°C	21 (28.3)
38-38.9°C	41 (55.4)
> 39°C	12 (16.3)
Pharyngitis	34 (45.9)
Tachypnea	45 (60.8)
Rhonchi	43 (58.1)
Cyanosis	10 (13.5)
Of patients needing intensive care	14 (18.9)
CPAP (continuous positive airway pressure)	4 (5.4)
Mechanical ventilation	10 (18.9)
Length of stay in intensive care unit (day)	11.3 ± 8.8

Tablo 3. TNF-α, IL-1β and IL-6 levels in patients and control group

	Patients			
	Non-fatal mean (SD)	Fatal mean (SD)	Control group	p
TNF-α (pg/mL)	20.9 (47.8)	43.0 (43.2)	4.1 (2.3)	**p= 0.003**
IL-1β (pg/mL)	7.1 (18.6)	2.1 (1.9)	7.5 (8.8)	p= 0.657
IL-6 (pg/mL)	191.0 (507.9)	1074.1 (918.8)	36.1 (34.2)	**p< 0.001**

TNF: Tumor necrosis factor, IL: Interleukin.

DISCUSSION

Influenza pandemics are one of the most serious viral diseases that affect the whole world, and can cause important mortality and morbidity. The disease can affect anyone without discrimination of gender or age[4]. Similarly, there were patients of all ages in our study, and no significant difference between genders was present.

The role of inflammatory cytokines and chemokines in the pathogenesis of influenza was shown in human and animal experiments[2,5]. The role of cytokines in human H5N1 influenza disease accompanied by high mortality is well known, and it was also suggested that an increase in cytokine levels might have been responsible for the high mortality rate in the 1918 H1N1 pandemic[6,3].

During experimental influenza infection in animals models, it was shown that while IFN-α, IL-6, and TNF-α levels increased significantly correlated with disease severity, increases in IL-8 and IL-1 levels were less obvious[7]. In other studies with mice, although the protective role of TNF-α was shown, a significant increase in TNF-α was determined as a sign of fatal disease and poor prognosis[8,9]. Moreover, it was shown in patients with influenza that especially TNF-α and IL-6 levels were related to the clinical findings of the disease[10,11].

Similarly, in our study, TNF-α and IL-6 levels were found to be higher in patients with influenza than in the control group and were even higher in fatal patients. IL-1β levels were found to be lower in fatal patients. In the study carried out by Giamarellos-Bourboulis et al., it was suggested that a decrease in IL-1 level was related to the anti-inflammatory effect of IL-6[12].

Hagau et al. performed a study during the 2009-2010 influenza season with 21 patients with influenza

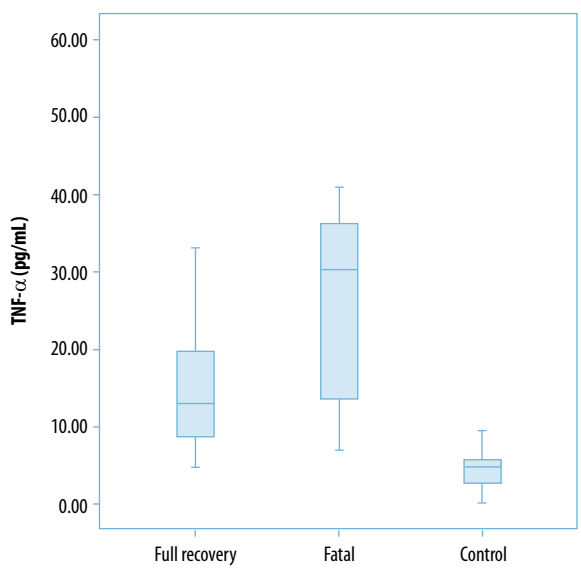


Figure 1

TNF-α results in the patients with full recovery, exitus patients and the control group.

Figure 2

IL-6 results in the patients with full recovery, exitus patients and the control group.

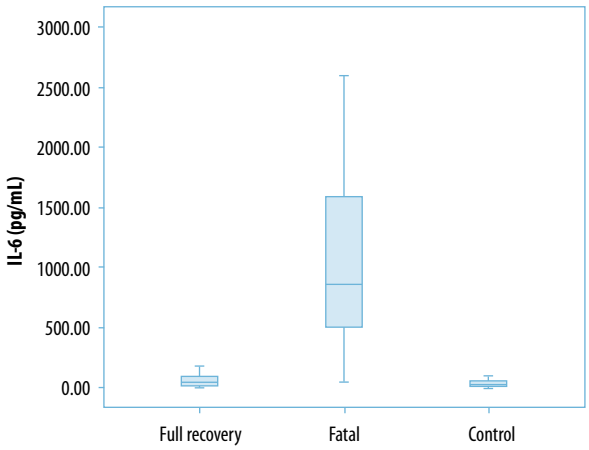
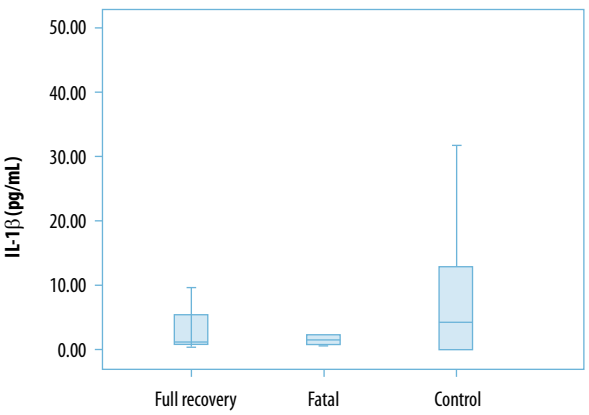


Figure 3

IL-1β results in the patients with full recovery, exitus patients and the control group.



A (H1N1)-related acute respiratory distress syndrome (ARDS), 11 patients with influenza A (H1N1) mild disease, and 15 healthy volunteers as a control group[13]. They found that the levels of IL-6, IL-8, IL-9, IL-12, IL-15, IL-10 and TNF-α were significantly increased in critically ill patients versus the control group. When mild and critical cases were compared, IL-6, IL-8, IL-15, and TNF-α were significantly higher in critical ARDS patients as hallmarks of disease severity. The higher levels of IL-6 and TNF-α in critically ill patients were similar to our results in fatal patients. Another study conducted by To et al. demonstrated that IL-6 levels were higher in those patients with more severe disease throughout the disease period, whereas IL-1α and TNF-α levels were higher only in the later phase of the disease[14]. In contrast to that study, we found higher TNF-α levels in the early phase of the disease, especially in the fatal patients.

The influenza A (H1N1) 2009 pandemic caused serious morbidity and mortality. Unlike the seasonal flu,

the pandemic influenza A (H1N1) 2009 caused mortality in patients without underlying risk factors. Therefore, it is important to predict in which patients the disease may be fatal. The significant increase in TNF-α and IL-6 levels in patients who did not survive demonstrated these markers to be useful as indicators of fatality. This study conducted among patients with H1N1 influenza is important in this respect, and we suggest that it could be important for the prediction of fatality.

Limitations of This Study

Due to limited resources during the pandemic, only hospitalized patients were sampled for RT-PCR test for the definitive diagnosis of influenza. Since there was no possibility for definitive diagnosis of the influenza, outpatients with mild disease were not included in this study.

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REFERENCES

1. *Zhang C, Xu Y, Jia L, Yang Y, Wang Y, Sun Y, Huang L, Qiao F, Tomlinson S, Liu X, Zhou Y, Song H. A new therapeutic strategy for lung tissue injury induced by influenza with CR2 targeting complement inhibitor. Virol J 2010; 7: 30.*
2. *Cheung CY, Poon LL, Lau AS, Luk W, Lau YL, Shortridge KF, Gordon S, Guan Y, Peiris JS. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? Lancet 2002; 360: 1831-7.*
3. *Kobasa D, Jones SM, Shinya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M, Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawaoka Y. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature 2007; 445: 319-23.*
4. *Kang YM, Song BM, Lee JS, Kim HS, Seo SH. Pandemic H1N1influenza virus causes a stronger inflammatory response than seasonal H1N1 influenza virus in ferrets. Arch Virol 2011; 156: 759-67.*
5. *Kim HM, Lee YW, Lee KJ, Kim HS, Cho SW, van Rooijen N, Guan Y, Seo SH. Alveolar macrophages are indispensable for controlling influenza viruses in lungs of pigs. J Virol 2008; 82: 4265-74.*
6. *Us D. Kuş gribinde sitokin fırtınası. Mikrobiyol Bul 2008; 42: 365-80.*
7. *Van Reeth K, Van Gucht S, Pensaert M. Correlations between lung proinflammatory cytokine levels, virus replication, and disease after swine influenza virus challenge of vaccination-immune pigs. Viral Immunol 2002; 15: 583-94.*
8. *Hofmann P, Sprenger H, Kaufmann A, Bender A, Hasse C, Nain M, Gemsa D. Susceptibility of mononuclear phagocytes to influenza A virus infection and possible role in the antiviral response. J Leukoc Biol 1997; 61: 408-14.*
9. *Conn CA, McClellan JL, Maassab HF, Smitka CW, Majde JA, Kluger MJ. Cytokines and the acute phase response to influenza virus in mice. Am J Physiol 1995; 268: 78-84.*
10. *Hayden FG, Fritz R, Lobo MC, Alvord W, Strober W, Straus SE. Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. J Clin Invest 1998; 101: 643-9.*
11. *Kaiser L, Fritz RS, Straus SE, Gubareva L, Hayden FG. Symptom pathogenesis during acute influenza: interleukin-6 and other cytokine responses. J Med Virol 2001; 64: 262-8.*
12. *Giamarellos-Bourboulis EJ, Raftogiannis M, Antonopoulou A, Baziaka F, Koutoukas P, Savva A, Kanni T, Georgitsi M, Pistiki A, Tsaganos T, Pelekanos N, Athanassia S, Galani L, Giannitsioti E, Kavatha D, Kontopidou F, Mouktaroudi M, Poulakou G, Sakka V, Panagopoulos P, Papadopoulos A, Kanellakopoulou K, Giamarellou H. Effect of the novel influenza A (H1N1) virus in the human immune system. PLoS One 2009; 4: e8393.*
13. *Hagau N, Slavcovici A, Gonganau DN, Oltean S, Dirzu DS, Brezoszki ES, Maxim M, Ciuce C, Mlesnite M, Gavrus RL, Laslo C, Hagau R, Petrescu M, Studnicska DM. Clinical aspects and cytokine response in severe H1N1 influenza A virus infection. Crit Care 2010; 14: R203.*
14. *To KK, Hung IF, Li IW, Lee KL, Koo CK, Yan WW, Liu R, Ho KY, Chu KH, Watt CL, Luk WK, Lai KY, Chow FL, Mok T, Buckley T, Chan JF, Wong SS, Zheng B, Chen H, Lau CC, Tse H, Cheng VC, Chan KH, Yuen KY. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin Infect Dis 2010; 50: 850-9.*

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