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**ORIGINAL ARTICLE**

# Serum tumor necrosis factor alpha and interleukin-4 levels in patients with Crimean-Congo Hemorrhagic Fever

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**ABSTRACT**

**Objectives:** Crimean-Congo hemorrhagic fever (CCHF) is a tick borne zoonotic infection. Up till now knowledge about pathogenesis of the disease is limited. Cytokine response is quite important during this infection. The aim of this study was to determine the serum tumor necrosis factor-alpha (TNF-α) and the interleukin-4 (IL-4) levels in patients with CCHF and compare with those of healthy controls.

**Materials and methods:** Forty-two adult CCHF patients and 40 healthy subjects were included in this study. The diagnosis of CCHF was based upon anti-CCHF virus IgM positivity (ELISA method), and/or positivity in real time-polymerase chain reaction (RT-PCR) in acute phase serum samples. Serum TNF-α and IL-4 levels were determined by ELISA method.

**Results:** In patients with CCHF the mean serum TNF-α level was significantly higher than the control group (15.01 vs. 7.69 pg/ml; $p<0.05$) and also the mean serum IL-4 levels were significantly higher than the control group (1.07 vs. 0 pg/ml; $p<0.05$).

**Conclusion:** The levels of serum TNF-α and IL-4 in CCHF patients were found higher than healthy controls. These cytokines might play an important role in the pathogenesis of CCHF. *J Microbiol Infect Dis 2011;1 (3):134-137*

**Key words:** Crimean-Congo Hemorrhagic Fever, IL-4, TNF-α

## Kırım-Kongo kanamalı ateşi hastalarında serum tümör nekroz faktör-alfa (TNF-α) ve interlökin-4 (IL-4) düzeyleri

**ÖZET**

**Amaç:** Kırım-Kongo kanamalı ateşi (KKKA) kene kaynaklı zoonotik bir enfeksiyondur. Günümüzde hastalığın patogenezi konusunda bilgiler sınırlıdır. İnfeksiyonların seyri sırasında sitokin yanıtı önemlidir. Bu çalışmanın amacı KKKA'lı hastalarda serum tümör nekroz faktör-alfa (TNF-α) ve interlökin-4 (IL-4) düzeylerini belirlemek ve sağlıklı kontrol grubu ile karşılaştırmaktır.

**Gereç ve Yöntemler:** Çalışmaya KKKA tanısı konan 42 hasta ile 40 sağlıklı gönüllü dahil edildi. KKKA tanısı akut dönemde serumda anti-CCHF virüs IgM pozitifliği (ELISA yöntemi) ve/veya real time-polimeraz zincir reaksiyonu (RT-PZR) pozitifliği ile konuldu. Serum TNF-α ve IL-4 düzeyleri ELISA yöntemiyle çalışıldı.

**Bulgular:** KKKA'lı hastalarda ortalama serum TNF-α seviyesi kontrollere göre istatistiksel olarak anlamlı şekilde daha yüksek bulundu (15,01'e karşılık 7,69 pg/ml; $p<0,05$). Ortalama serum IL-4 düzeyleri de hasta gurubunda kontrollere göre anlamlı oranda yüksek (1,07'ye karşılık 0 pg/ml; $p<0,05$) idi.

**Sonuç:** KKKA'lı hastalarda serum TNF-α ve IL-4 düzeylerinin yüksek olması bu sitokinlerin hastalık patogenezinde önemli rol oynayabileceğini göstermektedir.

**Anahtar kelimeler:** Kırım-Kongo kanamalı ateş, IL-4, TNF-α

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## INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF) is one of the viral hemorrhagic fevers that characterized by thrombocytopenia, fever and in most severe cases hemorrhage and shock. It is an important zoonotic infection and transmitted mainly by exposure to the Hyalomma ticks. CCHF virus, an enveloped RNA virus with helical symmetry, is the infectious agent of the disease and is a member of Nairovirus genus, belonging to the Bunyavirus family.[1-4] The disease was seen in various geographical regions in the world and CCHF outbreaks have been reported in Turkey, Bulgaria, Macedonia, Pakistan, Afghanistan, Iraq, Iran, Kosovo, Kazakhstan, some of the African countries, Caucasia, Russia, Yugoslavia, Greece and Kuwait. Until now, thousands of CCHF cases have been reported in CCHF outbreaks in Turkey.[5-8]

On the other hand, knowledge of the pathogenesis of the disease is limited. According to common knowledge, inflammatory processes have an important role during the infection. The role of T cells in the inflammatory processes of inflammatory response during CCHF course could be important. As known, T cells are divided into two groups according to the type of cytokines they release. Cytokines released from these cells are necessary for the immune response of the body against invading microorganisms. Th1 cells release pro-inflammatory cytokines such as TNF-α and interferon-γ (IF-γ) while Th2 cells release anti-inflammatory cytokines such as interleukin-10 (IL-10) and IL-4.[9,10] There is limited number of reports in the literature concerning cytokine response in CCHF.[11-13] Therefore we investigated the levels of serum IL-4, and TNF-α in CCHF patients.

## MATERIALS AND METHODS

In a prospective approach, a study was conducted in Ankara Education and Research Hospital. Forty-two adult patients diagnosed with CCHF based on history, clinical and laboratory findings and 40 healthy controls were included in the study. These control subjects did not have any chronic illness and were not affected by any acute medical problem at the time of the study. Ethical Committee approval was obtained from the Ethical Committee of Ankara Education and Research Hospital, and informed consent forms were signed by patients and control groups.

Serologic and virologic analyzes were performed in Refik Saydam Hygiene Center, Virology Referance Laboratory in Ankara. The diagnosis of CCHF was based upon anti-CCHF virus IgM positivity (ELISA method), and/or positivity in real time-polymerase chain reaction (RT-PCR) in acute phase serum samples. Serum TNF-α and IL-4 levels were determined by using commercial test kits (ELISA method, BioSource, Nivelles, Belgium) according to the instructions of the manufacturer. These tests were performed in Refik Saydam Hygiene Center, Virology Referance Laboratory in Ankara. All data of CCHF patients and healthy controls were entered into and used the Statistical Package for Social Science (SPSS), windows based software (version 11.5). Student's t and Chi-square tests were used in the statistical analyzes and statistical significance was defined as a two-tailed p value of $< 0.05$.

## RESULTS

In total 42 patients with CCHF and 40 healthy controls were included into the study. The mean age of CCHF cases was 34 years (± 4.2 years) and was 43 years (± 3.5) in the controls ($p>0.05$). In the cases with CCHF 25 (59.5) of them are male and in controls 22 (55.0%) are male ($p=0.05$). In patients with CCHF, the mean serum TNF-α level was significantly higher than the control group (15.01 vs. 7.69 pg/ml; $p<0.05$) and also the mean serum IL-4 levels were significantly higher than the control group (1.07 vs. 0 pg/ml; $p<0.05$).

## DISCUSSION

Clinical symptoms of the disease appear as a result of hepatocyte and endothel damage and the dramatic reduction in the number of platelets.[1-3,4-8] The pathogenesis of CCHF has not been well described. However, it has been hypothesized that it could be a result of the direct injury of virus-infected tissues in combination with the indirect effects of host immune responses, including cytokines.[13]

In many viral and bacterial infections, T cells involved with cellular immune response and cytokines, lymphokines and chemokines are released from these cells and their interactions play an important role against viral and bacte-

rial infections including tuberculosis and brucellosis.[9,10,14] Human immunodeficiency virus (HIV), chronic hepatitis B and C, severe acute respiratory syndrome (SARS), CCHF and Dengue Fever (DF) are some of the viral infections.[11,12,15-20] Cytokines may be classified as pro-inflammatory (e.g., TNF-α and IL-12) or anti-inflammatory (e.g., IL-4 and IL-10) according to their functions in inflammation. In hepatitis B infection, Th1/Th2 balance is important in the control of viral replication and emergence of liver damage. Th1 cells help cytotoxic T cells with the cytokines they release, and cytokines try to control viral replication.[20] Cytokines have been demonstrated to play a role both in pathogenesis and progression of infectious diseases. High levels of TNF-α occur in many diseases and it may be used in clinical monitorization of them.[10,16-19] In addition, IL-4 is also involved with the pathogenesis of various diseases and may be used in the follow-up of some diseases.[9,11,12,16,17,20] Akpolat et al., reported that IL-4 and IF-γ were influential in chronic progression while transforming growth factor beta 1 (TGF-β1) plays a role in the development of fibrosis in patients with chronic hepatitis B. Bal et al. demonstrated that in patients with co-infected with HIV and tuberculosis both Th1 and Th2 cells suppressed and TNF-α and IL-4 levels decrease accordingly.[16,17] The serum IL-6 levels were also found to be high in the same study. They concluded that decreased cytokine secretions might be attributed to deep immune suppression in the responses of Th1 and Th2 cells.

Fewer studies are available in the literature concerning cytokine response in CCHF. As in other VHFs including DF and yellow fever, cytokines play an important role in the pathogenesis and the clinical course of CCHF.[11,12] Papa et al. reported that higher levels of serum TNF-α and IL-6 in CCHF patients.[11] In that study, IL-10 levels were found to be higher in fatal cases. They also reported that TNF-α was associated with the severity of the disease while IL-6 increased both in mild and severe cases. In Turkey, Ergonul et al. showed that serum TNF-α and IL-6 levels correlated positively, while IL-10 levels correlated negatively with disseminated intravascular coagulation (DIC) scores in 3 fatal, 27 non-fatal 30 CCHF cases.[12] Higher levels of serum TNF-α and IL-4 in our CCHF cases are comparable with the results of Papa and Ergonul. In another study performed in Kosovo in 46 CCHF cases demonstrated weaker antibody response against CCHF virus, higher levels of IL-10, IF- γ and TNF-α were associated with poor outcome.[13] Besides, it was reported that a positive linear dependence between viral load and these cytokines was observed. In that study, lower levels of IL-12 were detected in all CCHF patients.

The authors hypothesized that CCHF could be a result of a delayed and down-regulated immune response caused by IL-10, which leaded to an increased replication of CCHFV throughout the body. They concluded consequently to increased production of IF-γ and TNF-α mediated vascular dysfunction, DIC, organ failure, and shock. In our study, as it was not possible to have access to the clinical information on the patients and treatment results, the relation between cytokine levels and clinical condition could not be established. We accept it is the restriction part of this study.

In conclusion, observed higher levels of serum TNF-α and IL-4 levels in our study show that cellular immunity including Th1 and Th2 cells responses could play an important role in the pathogenesis of CCHF.

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