Arch Clin Infect Dis. 2016 July; 11(3):e28029. doi: 10.5812/archcid.28029.

Published online 2016 June 26. **Research Article**

Evaluation of Serum Level of Interleukin-6 in Patients With Crimean-Congo Hemorrhagic Fever in Zahedan, Iran, From 2012 to 2015

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Received 2015 February 17; **Revised** 2016 May 16; **Accepted** 2016 May 29.

Abstract

Background: Crimean-Congo hemorrhagic fever (CCHF) is caused by a tick-borne virus in the family of Bunyaviridae. It is asymptomatic in infected animals, but it can cause severe hemorrhagic disease in human with mortality rate of 3% to 50%. Viral load and inflammatory cytokines have an important role in the pathogenesis of the disease.
Objectives: The current study aimed to investigate the level of interleukin (IL)-6 in patients with CCHF and the relationship of its level with the severity of infection and clinical outcome of patients with CCHF.
Patients and Methods: This cross-sectional and prospective study evaluated all patients with confirmed CCHF admitted to Boo-Ali hospital in Zahedan, Southeast of Iran, from March 2012 to Jan 2015. The disease was confirmed by enzyme-linked immunosorbent assay (ELISA) IgM and IgG and /or real time polymerase chain reaction (RT-PCR). Also, a blood sample was drawn on the day of admission to test the level of IL-6 in the serum of patients. Then, the relationship between serum level of IL-6 with the severity of disease and clinical outcome in patients was evaluated. Data was analyzed by Mann-Whitney test and a $P < 0.05$ was considered significant.
Results: Among 50 patients with CCHF (38% female, 62% male with the age range of 18 - 63 years), the serum level of IL-6 was higher than normal (7 pg/mL) in 60% of the patients. According to the severity index disseminated intravascular coagulation score (DIC score), the mean serum level of cytokine in the mild and severe disease were 50.2 ± 79.4 and 127.3 ± 100.2, respectively. There was a significant difference between serum level of IL-6 and severity of disease ($P = 0.003$). There was no mortality in patients with low or normal serum levels of IL-6. But, in patients with a high serum level of IL-6, seven patients died.
Conclusions: Based on the obtained results, it was concluded that a diminished immune response caused by cytokines in acute phase response, can lead to dissemination of virus, severity of illness and increase of the mortality rate.

Keywords: Crimean-Congo Hemorrhagic Fever, Clinical Outcome, Interleukin-6, Mortality, Severity

1. Background

Crimean-Congo hemorrhagic fever causes by a virus which is a member of the genus *Nairovirus* of family Bunyaviridae (1, 2). This virus is widespread in Africa, Asia, the Middle-East and in some parts of Europe such as Bulgaria, Greece, Albania, Hungary and Portugal. The occurrence of CCHF is related to the distribution of Hyalomma tick that acts as the principal vector in the transmission of virus. Changes in climatic conditions can influence the range of illness in the community (3-6). Human can be infected by tick bite or by contact with the infected animal blood or tissues, and sometimes drinking unpasteurized milk when an infected animal is in viremic phase. Human-to-human transmission is reported. This happens when skin or mucous membranes are exposed to the infected blood during hemorrhages. Aerosol transmission was reported as nosocomial transmission in Iran and Russia (7-11). Infected animals are usually asymptomatic, but the disease can cause a severe human disease with high mortality rate. The case-fatality rate of CCHF depends on the transmission route and geographic area, but can be as high as 50% (2, 4, 5, 12). Major signs of severe disease such as hemorrhage and severe thrombocytopenia are accompanied with increased vascular permeability that indicates the involvement of endothelial cells (ECs). CCHF virus can activate endothelial cells directly or indirectly by cytokines released from infected leukocytes. Activation of endothelial cells and destruction in endothelial barrier can lead to increased vascular permeability and this phenomenon initiates inflammatory responses (13-16). Releasing the pro-inflammatory cytokines such as IL-1, IL-6, IL-10, and tumor necrosis factor-α (TNF-α) from endothelial cells are the main factors in the pathogenesis of CCHFV. A diminished immune response

caused by cytokines such as IL-6 on the first days of disease can lead to severe infection. Many studies showed the role of cytokines in the pathogenesis of virus and severity and clinical outcome of the disease (17-22).

2. Objectives

Southeastern Iran is an endemic area for CCHF. Since 1999, several epidemics occurred in this area. The current study aimed to investigate the role of interleukin (IL)-6 in the severity of infection and clinical outcome of patients with Crimean-Congo hemorrhagic fever.

3. Patients and Methods

3.1. Design and Setting

The current cross-sectional and prospective study evaluated all patients with confirmed CCHF admitted to Boo-Ali hospital (a specialist hospital of infectious diseases and also a referral hospital in Zahedan, the center of Sistan and Baluchistan province, Southeast of Iran with 75 beds) from March 2012 to Jan 2015.

3.2. Participants and Sampling

First, 81 cases of patients with CCHF were studied. Exclusion criteria were: people who had used immunosuppressive drugs or had an immunodeficiency disorder; those who had used antiviral, steroids, or blood products; patients who had a negative test for CCHF. Totally, 31 patients were excluded from the present study. Inclusion criteria were: age $\geq$ 18 years and confirmed diagnosis of CCHF; finally, 50 patients were included in the study.

3.3. Measures and Measurements

When a patient with clinical signs and symptoms of CCHF was admitted to the hospital, blood samples were taken to evaluate CCHF on the days 0, 5 and 10. All serum samples were sent to the reference laboratory in Tehran, Iran. Also, a blood sample was drawn on the day of admission to test the level of IL-6. The disease was confirmed by enzyme-linked immunoassay (ELISA) IgM and IgG and /or real-time reverse transcription polymerase chain reaction (RT-PCR). Then, the relationship between serum level of IL-6 with the severity of disease and clinical outcome of patients with confirmed illness according to disseminated intravascular coagulation score (DIC score) was evaluated (19).

3.4. Statistical Analysis

Data were collected by one of the researchers of this work. Categorical variables (age, gender and severity of illness) were presented as counts and percentages. Data were analyzed by sample T-test and Mann-Whitney test. SPSS version 20 statistical software package (Chicago, IL) was used for data analysis. $P < 0.05$ was considered significant.

3.5. Codes of Ethics

A written informed consent including: demographic information, level of IL-6, and clinical outcome was obtained from each participant. The study was approved by ethical committees of Zahedan University of Medical Sciences under the code 17.

4. Results

Among 50 patients with confirmed CCHF (38% female, 62% male with the age range 18 - 63 years), the serum level of IL-6 was higher than normal in 60% of the patients. According to the severity index (DIC Score) (19), 68% of the patients (34 cases) had severe disease and 32% mild disease. The mean serum levels of cytokine in mild and severe diseases were 50.2 ± 79.4 and 127.3 ± 100.2, respectively. There was a significant difference between serum level of IL-6 and severity of disease ($P = 0.003$). There was no differences between gender and severity of illness ($P = 0.777$). There was no mortality in patients with low or normal serum level of IL-6. But, in the patients with a high serum level of IL-6 who had severe disease, seven cases died.

5. Discussion

Based on the current study results, a diminished immune response caused by cytokines such as IL-6 in acute phase response, can lead to spread of virus, severity of illness and increase of the mortality rate. Although, the pathogenesis of CCHFV is not well understood, it is reported that this virus impairs the cells that begin the antiviral immune response, lead to disseminated infection and spread virus and vascular dysregulation (13-20). On the other hand, previous studies reported that a viral load more than 108 copies/mL is a strong prognostic factor to differentiate between patients with CCHF who died from the ones who survived (20, 23). Interesting findings in previous studies were significantly increased level of IL-6 and IL-10 and reduced serum levels of IL-12 in all patients with

severe CCHF (17-20). IL-12 is an important inductor of cell-mediated immunity and is downregulated by IL-6 and especially IL-10. There are also similar reports with high production of both IFN-γ and TNF-α and anti-inflammatory cytokines such as IL-10 and IL-6 and low levels of IL-12 in patients with dengue hemorrhagic fever (DHF) and dengue hemorrhagic shock, which cause plasma leakage and an increase in microvascular permeability (23-25). Another study showed that resting endothelial cell had a low production of IL-6 and infected ECs with CCHFV had significantly increased levels of IL-6 ($P < 0.01$ and $P < 0.001$ at 24 to 72 hours post infection, respectively) (21). Previously, it was shown that CCHFV lead to releasing IL-6, IL-8, and TNF-α from infected dendritic cells and macrophages which implicate as important markers of severity in patients with CCHF (18-21). Increased serum levels of the interleukin-6, TNF-α and IL-8 in patients with severe form of CCHF disease were reported by researchers in Greece (26). This study reported that any patient who died had an increased serum level of IL-8. Ergonul et al. (21), in Turkey, studied the role of cytokines in the mortality of patients with CCHF. Serum levels of cytokines were measured in patients with fatal outcome and in patients with non-fatal illness. Levels of interleukin-6 were higher in patients with fatal disease than in patients with non-fatal illness ($P < 0.001$). Serum levels of IL-6 and TNF-α were positively correlated with DIC scores. They also found that the serum levels of IL-10 were not significantly different between fatal and non- fatal patients ($P = 0.937$). DIC score was also higher in the patients with fatal illness ($P = 0.023$) (21). Their findings explained that IL-6, IL-8 and TNF-α had the main role in the severity and mortality of patients with CCHF. These findings were similar to those of the current study results in present study. The current study demonstrated a significant difference between serum level of IL-6 and severity of disease ($P = 0.003$). In the current study, there was no mortality in patients with low or normal serum levels of IL-6. Death happened in seven patients with high serum levels of IL-6 who had severe disease.

5.1. Conclusion

The current study showed that the serum levels of the proinflammatory cytokines IL-6 was higher in CCHF patients with severe and fatal disease and there was a significant difference between the levels of IL-6 and severity of disease. More studies are needed to evaluate the pathogenesis of CCHFV. More factors are certainly involved in the pathogenesis of this virus which are waiting to be evaluated. The weak points of the study were: almost 40% of the subjects were excluded and delay to confirm the disease in patients, since the results should be confirmed by Pasteur Institute in Tehran. The strong point was a good collaboration of patients and also staff to conduct this research.

Acknowledgments

Authors would like to thank all staff in Boo-Ali hospital in Zahedan and Pasteur Institute of Iran in Tehran, who helped to conduct this research.

Footnotes

Authors' Contribution: All authors had an equal role in the writing of paper.

Funding/Support: This project was approved and financially supported by infectious diseases and tropical medicine research center at Zahedan University of Medical Sciences, Zahedan, Iran.

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