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Original article

Determination of urine tumor necrosis factor, IL-6, IL-8, and serum IL-6 in patients with hemorrhagic fever with renal syndrome

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ABSTRACT

Objective: The aim of this study was to explore the role of cytokines in the pathogenesis of hemorrhagic fever with renal syndrome (HFRS).
Methods: Double-antibody sandwich ELISA was used to determine serum interleukin (IL)-6, urine tumor necrosis factor (TNF), IL-6, and IL-8 levels in 56 patients with HFRS.
Results: Serum IL-6, urine TNF, IL-6, and IL-8 concentrations in HFRS patients were significantly higher than those in the control group ($p < 0.001$). The concentrations increased at fever stage, then continued to increase during the hypotension stage and peaked at the oliguria stage. The concentrations of serum IL-6, urine TNF, IL-6, and IL-8 increased according to the severity of the disease, and differed greatly among different types of the disease. Serum IL-6 had remarkable relationships with serum specific antibodies. It was positively related to serum β2-microglobulin (β2-MG), blood ureanitrogen (BUN), and creatinine (Cr). Significant positive relationships were also found both between urine IL-6 and TNF, and between IL-6 and IL-8 ($r = 0.5768$, $p < 0.05$; $r = 0.3760$, $p < 0.01$).
Conclusion: TNF, IL-6, and IL-8 were activated during the course of the disease. IL-6 was associated with the immunopathological lesions caused by the hyperfunction of the humoral immune response. IL-6, IL-8 and TNF were involved in renal immune impairment. Determining them might, to a certain extent, be useful in predicting the prognosis and outcome of patients with HFRS.

Introduction

Recent studies have shown that immunomodulation abnormities have a significant role in hemorrhagic fever with renal syndrome (HFRS). The hyperfunction of humoral immune response causes excessive generation of antigen-antibody complexes, leading to secondary immune reaction. It also causes hypofunction of stimuli, increase in $CD8T^+$ cells, and cellular immunomodulation dysfunction.[1–3] The dynamic change of the concentrations of serum interleukin-6 (IL-6), urine tumor necrosis factor (TNF), IL-6, and IL-8 in patients

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with HFRS were detected by ELISA. The relationships of IL-6 with serum specific IgM and IgG antibodies, and with β2-microglobulin (β2-MG), respectively, were also analyzed to explore the role of IL-6 in the pathogenesis of HFRS.

Methods and patients

Subjects

The subjects were 56 inpatients with positive HFRS-specific IgM and IgG antibodies, admitted to the Department of Infectious Diseases of this hospital and of the Xi'an Infectious Disease Hospital from 1992 to 2003. This study was conducted in accordance with the Declaration of Helsinki, with approval from the Ethics Committee of the First Affiliated Hospital of the Medical School of the Xi'an Jiaotong University. An informed consent was obtained from all participants. The diagnosis and classification were made according to the standard adopted during the National Epidemic Hemorrhagic Fever Symposium held in Nanjing in 1986. From the day the patients were admitted to the hospitals, blood and urine samples were collected twice a day, and a total of 195 blood samples and 186 urine samples were obtained, which were stored at negative 20 °C. The control group consisted of 20 healthy blood donors. Their HFRS specific IgM and IgG antibodies and hepatitis virus infection indicators were all negative.

Reagents

IL-6, TNF, IL-8 monoclonal antibodies, standard samples, and negative controls were provided by Professor Jin Boquan from the Department of Immunological Teaching and Research of the Fourth Military University. Fluorescent splits were made by the Shanxi Preventive Medicine Research Medical Institute. Fluorescent indicators (isosulfocyanic acid) containing IgG and IgM extracted from sheep blood and β2-MG kits were provided by the Shanghai Vaccine and Serum Institute.

Methods

Total serum globulins were determined by biuret. Specific IgM and IgG were detected by indirect immune fluorescence,[4] and B2-MG by specific radiation fluorescence, according to the specifications of the kits. IL-6, IL-8, and TNF were determined by ELISA. The ELISA plate (96-well plate for ELISA reaction; Maxisorp, Denmark) was coated with 100 μL/well of diluted capture antibody, incubated at 4 °C for 48 hours. On the third day, the plate was removed from 4 °C and rinsed three times with wash buffer, for 5 min each wash step. The standard curve and samples were added with 100 μL/well and incubated at 37 °C for 2 h. After the plate was washed three times, diluted biotinylated detection antibodies were dripped with 100 μL/well, and incubated at 37 °C for 1 h. The plate was washed three times. ABTS was added with 100 μL/well, and incubated at 37 °C for 30 min. The absorption value (OD 410 nm) was read by microplate spectrophotometer.

Statistical analysis

Results were expressed by the mean and standard deviation. Sample mean comparison was made using Student's *t*-test, square analysis and linear relative analysis. A p-value <0.05 was considered significant.

Results

Characteristics of the participants

A total of 76 unrelated Chinese Han subjects (56 inpatients with HFRS, and 20 healthy control individuals) were included in the study. The patients were an average of 36.8 (18-61) years old, and consisted of 46 males and 10 females. The healthy controls had an average age of 34.9 (24∼55) years old, and 50% of them were males. All of the patients detected by RT-PCR were positive for viral RNA. In accordance with the 1986 Nanjing standard, ten cases were diagnosed as mild, 20 as moderate, 18 as severe, and eight as very severe. One of the very severe patients died of a lung infection, and the others survived after treatment.

Change of serum IL-6

In the fever stage, the concentration of serum IL-6 in patients with HFRS was higher than that in control group ($p<0.001$). The concentration of serum IL-6 continued to increase during hypotension stage, reached its peak during the oliguria stage, decreased significantly during the polyuria stage, and remained higher than the controls during the convalescent stage. Fifteen patients with HFRS were tested at the same time and showed the same pattern of change (Table 1).

Changes of urine IL-6, IL-8, and TNF

As shown in Fig. 1, the concentration of urine TNF in patients with HFRS increased remarkably during the fever stage, reached its peak during the hypotension stage, decreased during the oliguria stage, and declined significantly during the polyuria stage. It also showed that the concentrations of IL-6 and IL-8 increased during the fever stage, and continued to increase during the hypotension stage, reaching their peak during the oliguria stage. Therefore, serum IL-6 and urine IL-6 were parallel ($r=0.76$, $p<0.01$).

Table 1 – Dynamic change of serum IL-6 in patients with HFRS during each stage ($\bar{x} \pm s$).

Stage	Number of cases	IL-6 ($ng.L^{-1}$)	p-value
Normal control group	20	85 ± 25	
Fever stage	37	586 ± 125	<0.001*
Hypotension stage	26	1043 ± 217	<0.01*
Oliguria stage	54	1209 ± 258	<0.05*
Polyuria stage	50	372 ± 81	<0.005*
Convalescent stage	28	170 ± 36	>0.05

* *vs* control group.