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Elevated chemokine levels during adult but not pediatric Crimean–Congo hemorrhagic fever

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ABSTRACT

Background: Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne viral zoonosis. Clinical reports indicate the severity of CCHF is milder in children than adults. The chemokines are important chemo-attractant mediators of the host immune system.
Objectives: The main aim of the study was to identify whether or not there were any differences in chemokine levels between the pediatric and adult patients and control groups, and whether there was any correlation with disease severity.
Study design: The serum levels of select chemokines including chemokine (C-C) ligand 2 (CCL2), CCL3, CCL4, chemokine (C-X-C) ligand 8 (CXCL8), CXCL9, and granulocyte-colony stimulating factor (G-CSF) in 29 adult and 32 pediatric CCHF patients and in 35 healthy children and 40 healthy adult control groups were studied by flow cytometric bead immunoassay method.
Results: Great variability was detected in the serum levels of the chemokines for both the adult and pediatric patients and controls. With the exception of G-CSF, the median serum levels of CCL2, CCL3, CCL4, CXCL8, and CXCL9 were found to be significantly higher in the adult patients compared to adult controls (2364.7 vs. 761 pg/ml; 714.1 vs. 75.2 pg/ml; 88.6 vs. 25.5 pg/ml; 217.9 vs. 18.3 pg/ml; 875 vs. 352.2 pg/ml, respectively, $p < 0.0001$ for all comparisons). Among the chemokines the median CCL4 and G-CSF levels were significantly higher in the pediatric patients compared to pediatric controls (40.3 vs. 7.1 pg/ml, $p < 0.0001$; 0.1 vs. 0.1 pg/ml, $p = 0.049$, respectively).
Conclusion: The results of this study showed prominent chemokine raising in adult CCHF patients compared to children CCHF patients.

1. Background

Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne viral zoonosis caused by CCHF virus (CCHFV), a single-stranded RNA virus belonging to the genus *Nairovirus* within the Bunyaviridae family [1,2]. The disease presentation in humans can range from mild to severe, with the latter categorized in four consecutive phases: incubation, pre-hemorrhagic, hemorrhagic, and convalescence [3–5]. Case fatality rates (CFRs) range between 3% and 30% and have been reported to be as high as 70% [3].

The pathogenesis of CCHF is still poorly understood mainly because outbreaks occur infrequently and due to the lack of a suitable animal model. Recent studies have suggested that the hemorrhagic syndrome in patients is due to an immunopathologic event, rather than direct damage caused by the virus [4]. Many host factors such as innate immune system cells, cytokines,

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endothelium, coagulation system, and virus titers have been attributed to severity of the disease [6–11]. Although clinical reports from Turkey noticed that severity of CCHF is milder and CFR is lower in children than adults, our previous report showed no difference in a range of serum cytokine levels between the adult and pediatric CCHF patients and between the pediatric CCHF patients having moderate or severe clinical course of the disease, defined by modified Swanepoel's criteria [12,13].

The chemokines are important chemo-attractant mediators of the host's immune system. They have a vital role in cellular trafficking and migration of neutrophils, monocytes, macrophages, dendritic cells, natural killer (NK)-cells, CD4+ and CD8+ T- cells, and B-cells that protect the body from pathogens [14] .

2. Objectives

Although serum chemokine levels have been investigated in some viral hemorrhagic fevers (VHFs) such as a novel bunyavirus VHF in China [15], and Rift Valley fever (RVF) [16], they have not yet been investigated in CCHF. Therefore, we aimed to investigate the serum levels of some chemokines including chemokine (C-C motif) ligand 2 (CCL2), CCL3, CCL4, chemokine (C-X-C motif) ligand 8 (CXCL8), CXCL9, and granulocyte-colony stimulating factor (G-CSF) in adult and pediatric CCHF patients in order to identify any correlation in the chemokine levels between the patient groups and disease severity.

3. Study design

3.1. Patient selection and collection of serum samples

Serum samples were taken from 29 adult patients (16 male, 13 female) with a mean age of 41.7 ± (SD) 16.53 (range: 19–71), and 32 children (20 male, 12 female) with a mean age of 11.9 ± (SD) 4.75 (range: 0.5–18). The samples were collected and stored at −80 °C from patients admitted to Cumhuriyet University and Hacettepe University Faculty of Medicine, between 2010 and 2011. The initial diagnosis of CCHF was based upon the results of enzyme-linked immunoassay (ELISA) and/or by real- time reverse transcriptase-polymerase chain reaction (RT-PCR) [17] run on serum samples from patients presenting with symptoms of viral hemorrhagic fever, i.e., high grade of fever and thrombocytopenia and/or hemorrhagic manifestations (ecchymosis, purpura, petechiae, gastrointestinal bleeding, and epistaxis). Definitive diagnosis was made based on a positive test result for CCHFV specific IgM antibody and/or viral antigen at either the acute or the convalescent phase of the disease. The study was approved by the local Ethical Committee of Zekai Tahir Burak Hospital, Ankara, Turkey. A written informed consent was obtained from all patients or their relatives and healthy controls before performing the study. For comparison, negative control sera were taken from 40 healthy blood bank donor adults (28 male, 12 female) with a mean age of 42 ± (SD) 10.82 (range: 24–80) years, and from 35 healthy children (18 male, 17 female) with a mean age of 13.03 ± 3.21 (range: 7–18) years.

Disease severity for pediatric patients was classified according to the modified Swanepoel's criteria defined in our previous report [13]. According to these criteria, pediatric patients having melaena/haematemesis, somnolence, a white blood cell (WBC) count >10,000/mm^3 or <4,000/mm^3, platelet count ≤50,000/mm^3, aspartate transferase (AST) level ≥135 U/L, alanine transferase (ALT) level ≥90 U/L, activated partial thromboplastin time (aPTT) ≥44 s, and fibrinogen level ≤150 mg/dl were defined as "severe CCHF" when fulfilling three or more of the above criteria during the first five days of the disease. The other pediatric patients were classified as having mild or moderate CCHF disease. Adult CCHF patients were classified using Swanepoel's criteria [5]. Cases were labeled severe if one of the following laboratory values occurred within the first five days of the disease: WBC count ≥10^4/mm^3, platelet count

Table 1
Children patients' clinical specifications.

Patient	Sex	Group	Age	Sample day	Patient clinical status
1	Male	Child	0	5	Mild/moderate
2	Male	Child	1	6	Mild/moderate
3	Male	Child	4	1	Mild/moderate
4	Male	Child	4	2	Mild/moderate
5	Female	Child	7	6	Mild/moderate
6	Male	Child	8	5	Mild/moderate
7	Female	Child	9	3	Mild/moderate
8	Male	Child	10	3	Mild/moderate
9	Female	Child	10	4	Mild/moderate
10	Female	Child	10	7	Severe
11	Female	Child	10	7	Mild/moderate
12	Male	Child	11	2	Mild/moderate
13	Male	Child	11	3	Mild/moderate
14	Female	Child	13	6	Severe
15	Male	Child	14	7	Mild/moderate
16	Female	Child	14	7	Severe
17	Male	Child	14	1	Mild/moderate
18	Male	Child	14	6	Mild/moderate
19	Male	Child	14	3	Mild/moderate
20	Male	Child	14	5	Mild/moderate
21	Male	Child	14	5	Mild/moderate
22	Male	Child	14	5	Mild/moderate
23	Male	Child	14	2	Mild/moderate
24	Male	Child	15	4	Mild/moderate
25	Male	Child	15	4	Mild/moderate
26	Female	Child	15	3	Severe
27	Male	Child	16	7	Mild/moderate
28	Female	Child	17	2	Mild/moderate
29	Female	Child	17	3	Mild/moderate
30	Female	Child	18	6	Mild/moderate
31	Male	Child	18	2	Mild/moderate
32	Female	Child	18	7	Severe