Journal of Clinical Virology 66 (2015) 76–82

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

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

Mehmet Arasli[a,*], Yasemin Ozsurekci[b], Nazif Elaldi[c], Alexander J. McAuley[d,e], Eda Karadag Oncel[b], Ishak Ozel Tekin[a], Mustafa Gokhan Gozel[c], Ali Kaya[f], Fusun Dilara Icagasioglu[f], Dilek Yagci Caglayik[g], Gulay Korukluoglu[g], Furuzan Kokturk[h], Mehmet Bakir[c], Dennis A. Bente[d,e], Mehmet Ceyhan[b]

[a] Department of Immunology, School of Medicine, Bulent Ecevit University, Zonguldak, Turkey
[b] Department of Paediatric Infectious Diseases, School of Medicine, Hacettepe University, Ankara, Turkey
[c] Department of Infectious Diseases and Clinical Microbiology, School of Medicine, Cumhuriyet University, Sivas, Turkey
[d] Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX, USA
[e] Department of Microbiology & Immunology, University of Texas Medical Branch,Galveston, TX, USA
[f] Department of Paediatrics, School of Medicine, Cumhuriyet University, Sivas, Turkey
[g] Refik Saydam National Public Health Agency, Virology Reference and Research Laboratory, Ankara, Turkey
[h] Department of Biostatistics, School of Medicine, Bulent Ecevit University, Zonguldak, Turkey

ARTICLE INFO

Article history:
Received 25 October 2014
Received in revised form 11 March 2015
Accepted 12 March 2015

Keywords:
Crimean–Congo hemorrhagic fever
Chemokine
Adults
Children

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,

* Corresponding author. Tel.: +90 538 683 8707; fax: +90 372 261 0264.
E-mail address: araslimehmet@yahoo.com (M. Arasli).

http://dx.doi.org/10.1016/j.jcv.2015.03.010
1386-6532/

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

Table 2
Adult patients' clinical specifications.

Patient	Sex	Group	Age	Sample day	Patient clinical status
1	Female	Adult	19	4	Mild/moderate
2	Male	Adult	19	3	Severe
3	Male	Adult	21	2	Mild/moderate
4	Male	Adult	21	2	Mild/moderate
5	Male	Adult	25	5	Mild/moderate
6	Male	Adult	26	7	Severe
7	Female	Adult	26	8	Mild/moderate
8	Female	Adult	27	7	Mild/moderate
9	Female	Adult	27	6	Severe
10	Male	Adult	27	5	Mild/moderate
11	Female	Adult	32	5	Mild/moderate
12	Male	Adult	33	1	Mild/moderate
13	Male	Adult	33	3	Severe
14	Female	Adult	34	5	Severe
15	Male	Adult	37	2	Mild/moderate
16	Male	Adult	44	6	Severe
17	Female	Adult	45	2	Severe
18	Female	Adult	54	8	Mild/moderate
19	Female	Adult	54	5	Mild/moderate
20	Male	Adult	55	3	Exitus
21	Female	Adult	56	3	Mild/moderate
22	Male	Adult	57	8	Exitus
23	Male	Adult	59	6	Mild/moderate
24	Male	Adult	59	6	Mild/moderate
25	Female	Adult	59	2	Severe
26	Male	Adult	61	5	Mild/moderate
27	Female	Adult	63	3	Mild/moderate
28	Female	Adult	64	7	Severe
29	Male	Adult	71	4	Mild/moderate

$\leq 2 \times 10^4/mm^3$, AST level ≥200 IU/L, ALT level ≥150 IU/L, and aPTT ≥60 s or fibrinogen level ≤110 mg/dl. The other adult patients were classified as having mild or moderate CCHF disease. Consequently, pediatric and adult CCHF patients were divided into two groups in this study: mild/moderate and severe.

3.2. Estimations of chemokines and G-CSF levels in serum samples

The acute phase serum levels of CCL2, CCL3, CCL4, CXCL8, CCXL9, and G-CSF were measured using a commercially available fluorescent bead immunoassay, Human Chemokine 6-plex FlowCytomix Multiplex kit (e-Bioscience, Vienna, Austria) according to the manufacturer's instructions. Briefly, fluorescent beads coated with monoclonal antibodies specific to distinct chemokines, together with chemokine-specific biotin-conjugated monoclonal antibodies were incubated with serum samples or serially diluted standards. After a 2-h incubation, the beads were washed twice and incubated with streptavidin–phycoerythrin for another hour. After repeating the washing procedure, samples were ready for flow cytometric analysis. Samples were then analyzed by using a Beckman Coulter Cytomics FC500 flow cytometry (Beckman Coulter Inc., Miami, FL, USA). The concentrations were measured by using Flow Cytomix Pro 3.0 software (e-Bioscience, Vienna, Austria).

3.3. Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS), version 18.0 software (SPSS Inc., Chicago, IL, USA). Distribution of data was determined by Shapiro–Wilks normality test. Continuous variables were expressed as median (range) and were compared with the Mann–Whitney *U* test. All tests were two tailed and a *p*-value of less than 0.05 was considered statistically significant for all tests. Graphs were plotted using Graphpad Prism, version 6.0 (Graphpad Software, Inc., La Jolla, CA, USA).

4. Results

Thirty two pediatric (Table 1) and 29 adult CCHF (Table 2) cases were enrolled. According to Swanepoel's criteria, eleven adults (37.9%) were classified as having severe CCHF disease, with two fatalities, and the remainder (62.1%) was defined as having mild/moderate CCHF disease. Within the pediatric group, according to the modified Swanepoel's criteria, five children (15.6%) were classified as having severe CCHF disease and 27 children (84.4%) were defined as having mild/moderate CCHF disease. There were no fatalities amongst the pediatric cases.

Great variability was seen in the serum levels of the studied chemokines for both the adult and the pediatric patients and controls. With the exception of G-CSF, the median serum levels of all of the chemokines were significantly higher in infected adults compared to healthy controls (Fig. 1). For CCL2, the median serum level of the chemokine in infected adults was 2364.7 (range: 436.8–28,181.6) pg/ml, 3.11 -fold greater than that of the controls [761.01 (199.5–3913.5) pg/ml; $p < 0.0001$]. For CCL3, the median serum level in infected adults was 714.1 (0.1–14,524.2) pg/ml, 9.49-fold greater than the median of the controls [75.2 (0.1–1410.3) pg/ml; $p < 0.0001$]. For CCL4, the median serum level in infected adults was 88.6 (6.7–1704.8) pg/ml, 3.48-fold greater than that of the controls [25.5 (3.2–89.4) pg/ml; $p < 0.0001$]. For CXCL8, the median serum level in infected adults was 217.9 (0.1–8917) pg/ml, 11.90-fold greater than that of the controls [18.3 (0.1–165.9) pg/ml; $p < 0.0001$]. The median serum level of CXCL9 in infected adults was 875 (469.7–9511.39) pg/ml, 2.48-fold greater than that of the controls [352.2 (62.8–3038.1) pg/ml; $p < 0.0001$]. Finally, the median level of G-CSF in the infected adults was 0.1 (0.1–258.7) pg/ml and it was 0.1 (0.1–280.7) pg/ml in controls ($p > 0.05$).

For the pediatric samples (Fig. 2), the median CCL4 level was 40.3 (0.1–2693.6) pg/ml in the infected patients and it was 7.1 (0.1–122) pg/ml in the pediatric controls ($p < 0.0001$) and fold change was 5.65. Despite the serum G-CSF levels were identical between the infected children and pediatric controls (0.1 pg/ml for both groups)

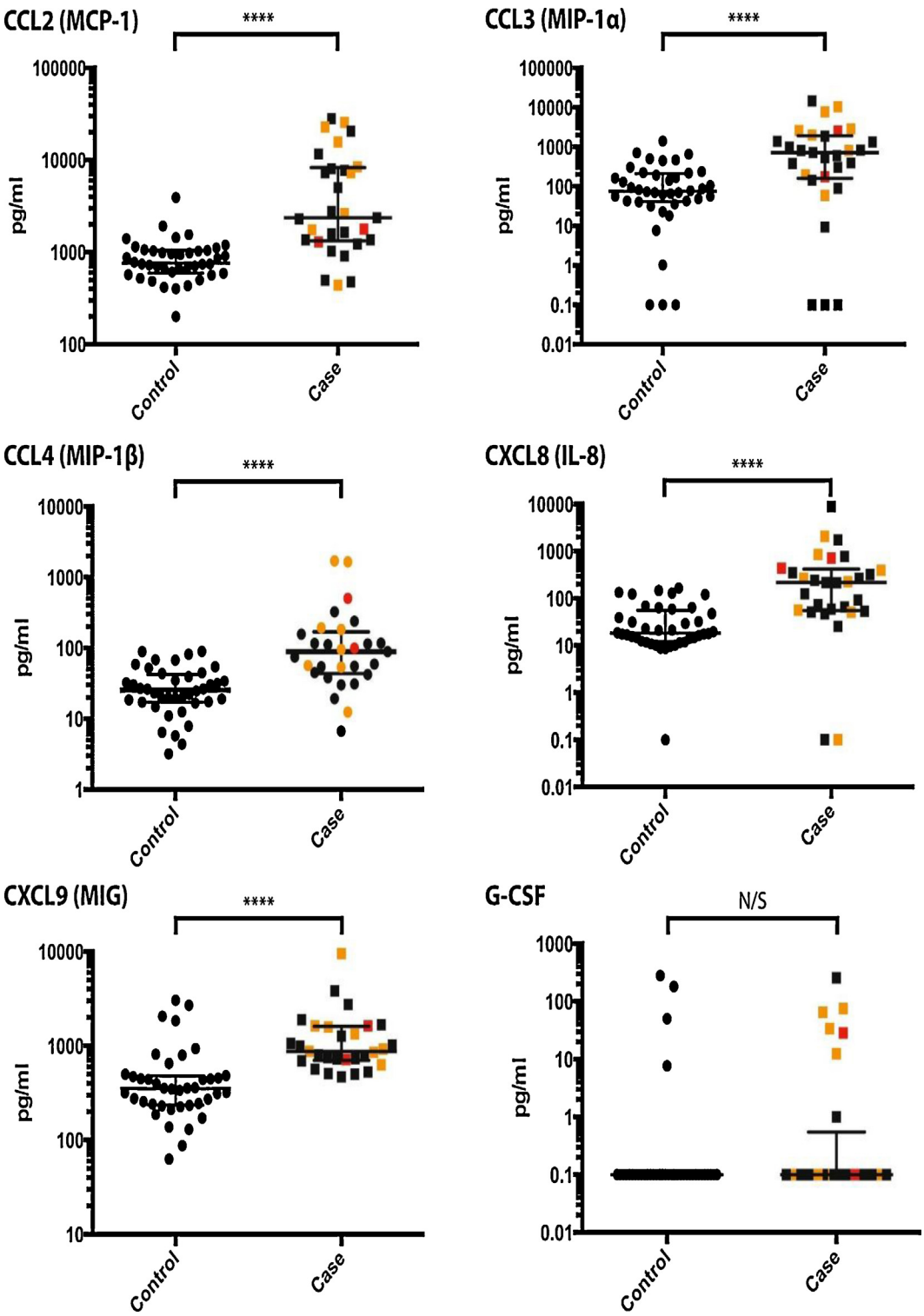

Fig. 1. Comparison of serum chemokine levels in CCHFV-infected adult patients (case) and uninfected healthy adults (control). Round black dots reflect healthy controls. Black squares denote CCHFV-infected adult patients while orange dots depict severe clinical presentation and red dots represent fatal cases. Median (black line) for each group as well as standard deviation was plotted. Groups were compared using Mann–Whitney *U*-test and values are considered statistically significant (****) when $p < 0.001$. N/S, not significant. The technical detection limit of each chemokine are the following ranges: 0– 30,000 pg/ml for CCL2, 0– 10,000 pg/ml for CCL3, 0– 3000 pg/ml for CCL4, 0– 10,000 pg/ml for CXCL8, 0– 5000 pg/ml for CXCL9, and 0– 25,000 pg/ml for G-CSF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ranges were different (0.1–944.5 vs. 0.1–257.3 pg/ml, respectively) and a statistically significant difference was observed between the groups ($p = 0.049$).

Further statistical analysis was also carried out to determine whether the presence of significant differences between the serum chemokine levels in patients with severe vs. mild/moderate infections (severe cases were marked in yellow and fatal in red in Figs. 1 and 2). No significant differences were detected for any of the chemokines in either the adult or pediatric groups (data not shown, $p > 0.05$ for all comparisons). In addition, no significant

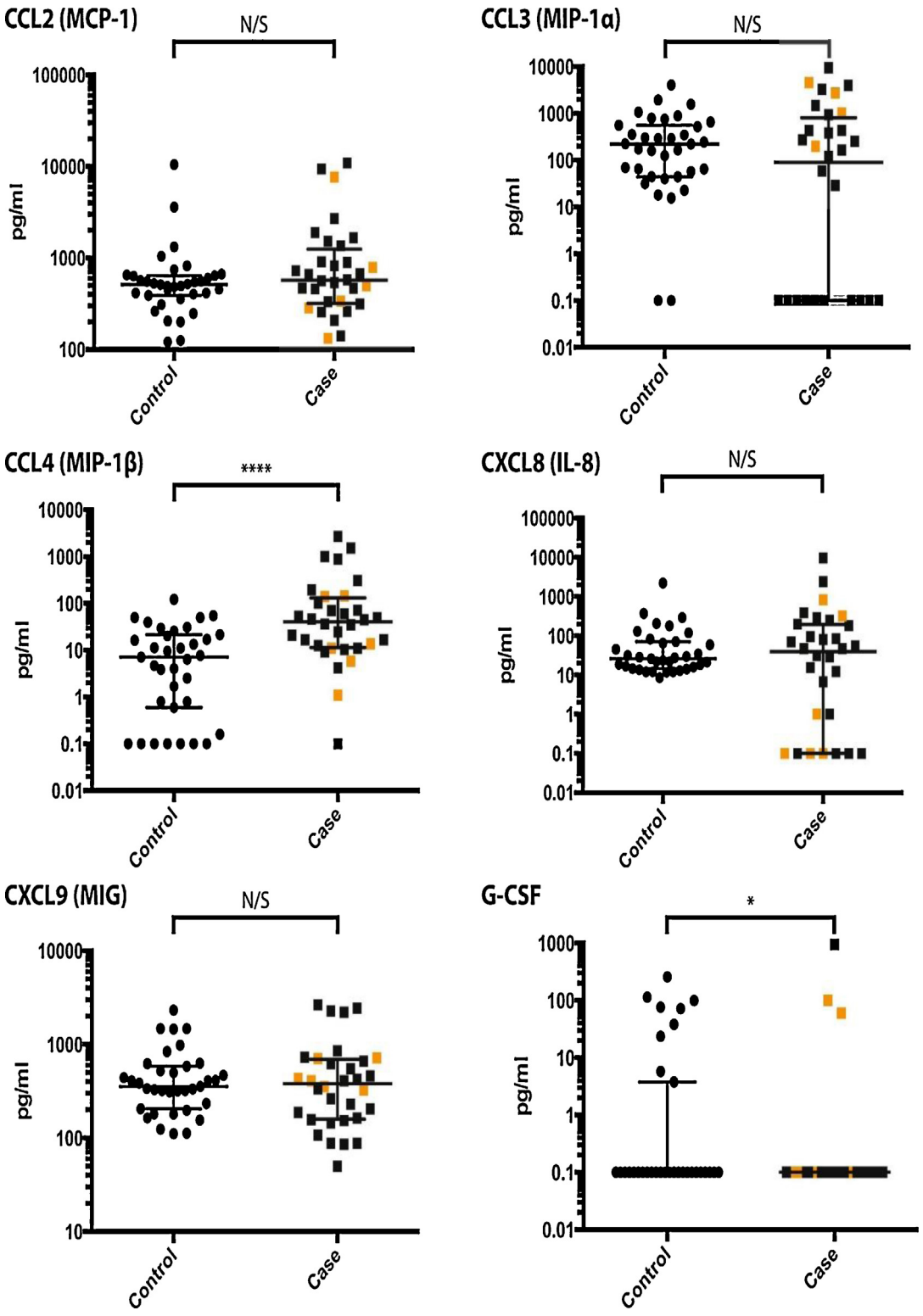

Fig. 2. Comparison of serum chemokine levels in CCHFV-infected pediatric patients (case) and uninfected healthy children (control). Round black dots reflect healthy controls. Black squares denote CCHFV-infected pediatric patients while orange dots depict severe clinical presentation. Median (black line) for each group as well as standard deviation was plotted. Groups were compared using Mann–Whitney *U*-test and values are considered statistically significant (*) when $p < 0.05$ and (****) when $p < 0.001$. N/S, not significant. The technical detection limit of each chemokine are the following ranges: 0– 30,000 pg/ml for CCL2, 0– 10,000 pg/ml for CCL3, 0–3000 pg/ml for CCL4, 0–10,000 pg/ml for CXCL8, 0– 5000 pg/ml for CXCL9, and 0– 25,000 pg/ml for G-CSF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

differences were also observed within the infected adult or pediatric groups when further classified into groups based upon age, gender or the time after onset of symptoms that the samples were taken (data not shown, $p > 0.05$ for all comparisons).

5. Discussion

Crimean–Congo hemorrhagic fever virus causes a hemorrhagic syndrome in humans that is characterized by dysregulated immune responses with high levels of pro-inflammatory cytokines that eventually cause vascular leakage and hypovolemic shock. Monocytes, macrophages, and dendritic cells (DCs) are considered the main targets for the virus [7,18]. Infection of DCs with CCHFV causes partial activation, leading to immune paralysis, whereas infection of macrophages and monocytes causes an overreactive secretion of cytokines [19]. Chemokines play important roles in both innate and adaptive immunity, especially for cell migration and trafficking. Very little is known about role they may play in CCHFV infection. Two in vitro studies, one looking at macrophages and DCs, the other looking at HUH7 cells, have been performed to date. It was demonstrated that DCs secreted high levels of IL-8 and macrophages secreted increased levels of CCL2, CCL3, IP-10, and RANTES [20]. The second study showed high levels of CXCL8 when HUH7 liver cells were infected with CCHFV. Interestingly, no other chemokines tested such as CCL3, CCL4 and IP-10, RANTES were increased [21] .

In this study, we demostrated that, with the exception of G-CSF, the serum levels of chemokines tested, namely including CCL2, CCL3, CCL4, CXCL8, and CXCL9, were significantly higher in adult patients compared to healthy controls.

CCL2 belongs to the CC chemokine family, which is mainly expressed by macrophages in response to LPS, IL-6, TNF-α, and IL-1β. This chemokine leads to monocyte, T lymphocyte, and NK cell infiltrations. Since monocytes, macrophages, and dendritic cells are the primary target cells for CCHFV, additional recruitment of susceptible cells to the site of virus replication is pathogenic mehanism that would benefit the virus and lead to higher virus titres in tissues and, consequently, more cytokine secretion. These findings are also support by the fact that high levels of CCL2 were also reported in an animal model for CCHFV infection [22].

In infected individuals, amount and composition of some chemokines are important factors in terms of T_H1 and T_H2 polarity. IFN-γ induces expression of CCL3 that recruits monocytes, neutrophils, and T_H1 lymphocytes, causes T_H1 differentiation. Conversely, IL-4 and IL-13 induce CCL2, leading to a T_H2 differentiation. Consequently, certain pathogens can determine the type of immune response by causing T_H1 and T_H2 polarity. IFN-γ and IL-4 can mutually counteract each other with regards to chemokine variety [23,24]. It is possible that the severe clinical course of adult patients with CCHF as compared with pediatric patients may be explained by this excessive CCL2 response. In our previous report, significantly higher serum levels of IL-13 were detected in the adult patients with fatal outcome when compared to the cytokine levels in patients who survived the infection [13]. Within the CC-chemokines family, CCL3 and CCL4 are highly homologous chemokines and use the same receptor, namely chemokine receptor (CCR)-5. These chemokines are also co-activators of macrophages. They have important roles in cellular and humoral immune responses and inflammation. Similar to CCL2, both CCL3 and CCL4 attract mononuclear cells [25]. Both chemokines are associated with type-1 immune responses. CCL3 has effect on the activation of naive CD4+ and CD8+ T-cells, B-cells and NK-cells. It has been reported that CCL3-deficient mice are more susceptible to viral infections, and T-cell dependent clearance of virus was found to be reduced in the model of infection [26,27]. Contrary to these reports, CCL3 levels were significantly lower in pediatric patients with CCHF, a group for whom the clinical course is often milder, than in adults in this study. It is clear that we are still far from understanding the various steps involved in T-cell dependent virus elimination. CXCL9 belongs to the CXC chemokines family. It binds to CXCR3 receptor, which is mainly expressed on activated T-cells and on a small subset of monocytes. The main function of this chemokine is specifically T-cell migration, and it plays an important role in T_H1-mediated antiviral activity [28]. CXCL9 is another chemokine demonstrated to be significantly higher in adult patients with CCHF than in pediatric patients in the present study. CXCL8 (IL-8) is chemoattractant for neutrophils, basophils, atopic eosinophils, T-cells, and B-cells [28]. It stimulates neutrophil transmigration from the blood vessels into tissues and activates degranulation and respiratory burst in neutrophils as well. It also inhibits IL-4 induced growth of B-cells and IgE production. It can be inferred from our findings that both T_H1 and T_H2 mediated antiviral response may play an important role simultaneously in the disease course of the CCHF patients.

Rift Valley fever (RVF) is a mosquito-borne VHF caused by RVF virus belonging to the family of Bunyaviridae. A weak pro-inflammatory cytokine response, both in serum and liver tissues, was reported in an experimental RVF infection in the C57BL/6 mouse model, whereas, the authors reported that CCL2, CCL3, and CXCL1 responses were significantly high. The elevated chemokine levels in that model were found to be correlated with liver histology showing cellular infiltration of T-cells, neutrophils, monocytes, and macrophages [29].

A newly defined VHF agent in the Bunyaviridae family, named Huaiyangshan virus (HYSV) and also known as severe fever with thrombocytopenia virus (SFTSV) is the cause of fever thrombocytopenia and leukopenia syndrome (FTLS). The virus is within the same family as CCHFV and an outbreak occurred with similar signs and symptoms of VHF among farmers in China. It has been demonstrated that there are higher levels of CXCL8, CCL2, and CCL4 in fatal cases than in non-fatal patient group or healthy individuals [15]. Although the severity of VHF is linked to high levels of chemokines in adults as mentioned above, the causal relationship between pathogenicity and chemokine response remains unclear in CCHF, according to the results of the present study.

The results of this study showed that the serum levels of chemokines related with CD4+ and CD8+ T-cells, B-cells, NK-cells, neutrophils, and macrophages are elevated during the acute phase of CCHF in adults, but not in children, suggesting that the prominent raising of the chemokines in adults may contribute to the more severe clinical course of the disease in this group.

Competing interest

The authors declare no competing interests or conflict of interest.

Funding

Human Chemokine 6-plex FlowCytomix Multiplex kit was supported by the authors.

Ethical approval

The ethical approval was given by The Republic of Turkey, Ministry of Health, Zekai Tahir Burak Women Health Education and Research Hospital Clinical Research Local Ethical Committee, Ankara, Turkey. (Date: 18th February 2014, Reference Number: 8/2014). A written informed consent was obtained from all patients or their relatives and healthy controls before performing the study.

References

[1] C.A. Whitehouse, Crimean–Congo hemorrhagic fever, Antiviral Res. 64 (2004) 145–160.
[2] O. Ergonul, Crimean–Congo hemorrhagic fever virus: new outbreaks, new discoveries, Curr. Opin. Virol. 2 (2012) 215–220.
[3] O. Ergonul, Crimean-Congo hemorrhagic fever, Lancet Infect. Dis. 6 (2006) 203–214.
[4] D.A. Bente, N.L. Forrester, D.M. Watts, A.J. McAuley, C.A. Whitehouse, M. Bray, Crimean–Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity, Antiviral Res. 100 (2013) 159–189.
[5] R. Swanepoel, D.E. Gill, A.J. Shepherd, P.A. Leman, J.H. Mynhardt, S. Harvey, The clinical pathology of Crimean–Congo hemorrhagic fever, Rev. Infect. Dis. 11 (suppl 4) (1989) S794–S800.
[6] A. Papa, S. Bino, E. Velo, A. Harxhi, M. Kota, A. Antoniadis, Cytokine levels in Crimean–Congo hemorrhagic fever, J. Clin. Virol. 36 (2006) 272–276.
[7] A.M. Connolly-Andersen, I. Douagi, A.A. Kraus, A. Mirazimi, Crimean–Congo hemorrhagic fever virus infects human monocyte-derived dendritic cells, Virology 390 (2009) 157–162.
[8] A.M. Connolly-Andersen, G. Moll, C. Andersson, et al., Crimean–Congo hemorrhagic fever virus activates endothelial cells, J. Virol. 85 (2011) 7766–7774.
[9] H. Bodur, E. Akinci, P. Ongürü, et al., Evidence of vascular endothelial damage in Crimean–Congo hemorrhagic fever, Int. J. Infect. Dis. 14 (2010) e704–e707.
[10] D. Duh, A. Saksida, M. Petrovec, et al., Viral load as predictor of Crimean–Congo hemorrhagic fever outcome, Emerg. Infect. Dis. 13 (2007) 1769–1772.
[11] A. Saksida, D. Duh, B. Wraber, I. Dedushaj, S. Ahmeti, T. Avsic-Zupanc, Interacting roles of immune mechanisms and viral load in the pathogenesis of Crimean–Congo hemorrhagic fever, Clin. Vaccine Immunol. 17 (2010) 1086–1093.
[12] M. Kızılgun, A. Ozkaya-Parlakay, H. Tezer, et al., Evaluation of Crimean–Congo hemorrhagic fever virus infection in children, Vector Borne Zoonotic Dis. 13 (2013) 804–806.
[13] Y. Ozsurekci, M. Arasli, E. Karadag Oncel, et al., Can the mild clinical course of Crimean–Congo hemorrhagic fever in children be explained by cytokine responses, J. Med. Virol. 85 (2013) 1955–1959.
[14] X. Blanchet, M. Langer, C. Weber, R.R. Koenen, P. von Hundelshausen, Touch of chemokines, Front. Immunol. 3 (2012) 175.
[15] Y.Z. Zhang, Y.W. He, Y.A. Dai, et al., Hemorrhagic fever caused by a novel Bunyavirus in China: pathogenesis and correlates of fatal outcome, Clin. Infect. Dis. 54 (2012) 527–533.
[16] A.K. McElroy, S.T. Nichol, Rift Valley fever virus inhibits a pro-inflammatory response in experimentally infected human monocyte derived macrophages and a pro-inflammatory cytokine response may be associated with patient survival during natural infection, Virology 422 (2012) 6–12.
[17] M. Yapar, H. Aydoğan, A. Pahsa, et al., Rapid and quantative detection of Crimean–Congo hemorrhagic fever virus by one-step real time reverse transcriptase-PCR, Jpn. J. Infect. Dis. 58 (2005) 358–362.
[18] T.W. Geisbert, L.E. Hensley, T. Larsen, et al., Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection, Am. J. Pathol. 163 (2003) 2347–2370.
[19] M. Bray, Pathogenesis of viral hemorrhagic fever, Curr. Opin. Immunol. 17 (2005) 399–403.
[20] C.N. Peyrefitte, M. Perret, S. Garcia, et al., Differential activation profiles of Crimean–Congo hemorrhagic fever virus- and Dugbe virus-infected antigen-presenting cells, J. Gen. Virol. 91 (2010) 189–198.
[21] R. Rodrigues, G. Paranhos-Baccalà, G. Vernet, C.N. Peyrefitte, Crimean–Congo hemorrhagic fever virus- infected hepatocytes induce ER-stress and apoptosis crosstalk, PLoS One 7 (1) (2012) e29712.
[22] D.A. Bente, J.B. Alimonti, W.J. Shieh, et al., Pathogenesis and immune response of Crimean–Congo hemorrhagic fever virus in a STAT-1 knockout mouse model, J. Virol. 84 (2010) 11089–11100.
[23] T.S. Olson, K. Ley, Chemokines and chemokine receptors in leukocyte trafficking, Am. J. Physiol. Regul. Integr. Comp. Physiol. 283 (2002) R7–28.
[24] A. Rot, U.H. von Andrian, Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells, Annu. Rev. Immunol. 22 (2004) 891–928.
[25] S.J. Allen, S.E. Crown, T.M. Handel, Chemokine receptor structure, interactions, and antagonism, Annu. Rev. Immunol. 25 (2007) 787–820.
[26] D.N. Cook, M.A. Beck, T.M. Coffman, et al., Requirement of MIP-la for an Inflammatory response to viral infection, Science 269 (1995) 1583–1585.
[27] T.P. Salazar-Mather, T.A. Hamilton, C.A. Biron, A chemokine-to-cytokine-to-chemokine cascade critical in antiviral defense, J. Clin. Invest. 105 (2000) 985–993.
[28] K.J. Laing, C.J. Secombes, Chemokines, Dev. Comp. Immunol. 28 (2004) 443–460.
[29] K.K. Gray, M.N. Worthy, T.L. Juelich, et al., Chemotactic and inflammatory responses in the liver and brain are associated with pathogenesis of Rift Valley fever virus infection in the mouse, PLoS Negl. Trop. Dis. 6 (2) (2012) e1529.