Jpn. J. Infect. Dis., 67, 22-26, 2014

Original Article

Plasma Oxidative Stress and Total Thiol Levels in Crimean-Congo Hemorrhagic Fever

Eda Karadag-Oncel[1]*, Ozcan Erel[2], Yasemin Ozsurekci[1], Dilek Yagci Caglayik[3], Ali Kaya[4], Mustafa Gokhan Gozel[5], Fusun Dilara Icagasioglu[4], Aynur Engin[5], Gulay Korukluoglu[3], Yavuz Uyar[3], Nazif Elaldi[5], and Mehmet Ceyhan[1]

[1]*Department of Pediatrics, Pediatric Infectious Disease Unit, Faculty of Medicine, Hacettepe University, Ankara;*
[2]*Department of Biochemistry, Atatürk Training and Research Hospital, Ankara;*
[3]*Refik Saydam National Public Health Agency, Ankara; and*
[4]*Department of Pediatrics and* [5]*Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey*

(Received April 4, 2013. Accepted August 2, 2013)

SUMMARY: In this study, we investigated the pro- and antioxidant status of patients with a pathogenesis of Crimean-Congo hemorrhagic fever (CCHF) in terms of their role in its pathogenesis. During the study period, 34 children and 41 adults were diagnosed with CCHF. The control group consisted of healthy age- and gender-matched children and adults. Serum levels of the total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), and plasma total thiol (TTL) were evaluated and compared between groups. The difference in mean TAC values between CCHF patients and healthy controls was not statistically significant ($P > 0.05$). Mean TOS, OSI, and TTL values were significantly lower in CCHF patients than in healthy controls ($P < 0.001$). Comparisons between the 2 groups revealed that mean TOS and OSI values were significantly lower in adults with CCHF than in their healthy counterparts ($P < 0.001$). Similarly, mean TTL levels were lower in both children and adults with CCHF when compared separately with healthy controls ($P < 0.05$). There was no significant difference in the mean serum TTL levels between children and adults with CCHF ($P > 0.05$). Our results suggest that TTL may play a more important role in CCHF pathogenesis than the other parameters investigated. The mean TOS and OSI values were higher in the control group than in CCHF patients.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal disease caused by CCHF virus (CCHFV), a member of the genus *Nairovirus* of the family *Bunyaviridae*. It is the most extensively spread tick-borne virus and the second most frequently encountered medically important arbovirus, after the dengue virus (1–3). Most cases of CCHF have been reported from parts of Africa, Asia, Eastern Europe, and the Middle East (1), with a mortality rate of 3%–30% (2). CCHFV is transmitted to humans either by ixodid tick bites (mostly of the *Hyalomma* genus), or contact with blood or tissues from CCHFV-infected patients or viremic livestock (4). Human CCHFV infections are usually characterized by a febrile illness accompanied by headache, myalgia, and a petechial rash. Because of its high fatality rate, CCHF remains one of the most important public health issues in Turkey. According to the epidemiological data from the Turkish Ministry of Health, more than 7,000 confirmed cases of CCHF were reported by the year 2012, with a 5% fatality rate (unpublished data). CCHFV infection runs a more severe and fatal clinical course in adults than in children, but the reasons behind these differences are yet to be elucidated (5).

Although CCHF pathogenesis remains poorly understood, a major target of CCHFV is widely believed to be the endothelium (6). In autopsies of deceased CCHF patients, viral antigens were detected in endothelial cells, and the density of molecular markers of endothelial activation was found to correlate with disease severity (7,8). Endothelial cell activation increases vascular permeability and initiates an inflammatory response by recruiting leukocytes via adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin. Endothelial/platelet-derived reactive oxygen species (ROS) enhance platelet activation and adhesion while simultaneously promoting coagulation during inflammation (9,10). ROS can increase both platelet adhesion to the endothelium (9) and platelet aggregation, promote translocation and upregulation of P-selectin at the surface of platelets and endothelial cells (10), and upregulate tissue factor expression (10,11). Similarities between ROS-induced vascular changes and changes observed in CCHF prompted us to investigate the potential role of ROS in the pathogenesis of this disease.

Following infection of the endothelium, viral or

*Corresponding author: Mailing address: Pediatric Infectious Disease Fellowship, Hacettepe University Ihsan Dogramacı's Child Hospital, Sıhhıye, Ankara, Turkey. Tel: +90 312 3051166, Fax: +90 312 3108241, E-mail: dredakaradag@gmail.com

virus-mediated host-derived soluble factors promote endothelial activation and dysfunction. Ensuing endothelial damage may lead to coagulopathy directly or via the dysregulation of platelet aggregation, which in turn activates the intrinsic coagulation cascade (12,13). This may explain why the clinical picture of CCHF includes vascular changes, thrombocytopenia, and disseminated intravascular coagulation, which may collectively contribute to the bleeding disorder associated with this disease (13). Reactive oxygen/nitrogen species, which develop as a result of endothelial damage, induce several structural and functional changes in hemostatic elements. Modifications of components of the hemostatic system, such as oxidation and nitration, have been observed in various inflammatory diseases associated with vascular complications (14). Persistence of inflammation is a contributing factor for maintaining a disturbed oxidative balance, which may be present in the form of increased oxidative stress and/or diminished antioxidant activities. Some studies have demonstrated that various inflammatory cells are activated in many infectious diseases, which result in the production of reactive oxygen and/or nitrogen species to kill microorganisms (15–17). Two different studies demonstrated a reduced total antioxidant capacity (TAC) and elevated total oxidant status (TOS) in brucellosis (17,18). The authors of these studies speculated that increased oxidative stress may cause severe oxidative damage in the body and found that this damage was ameliorated after antimicrobial treatment. Another study that investigated the oxidative status in Fasciola hepatica infections showed that plasma TOS levels and the oxidative stress index (OSI) were significantly increased in infected patients than in healthy controls. In contrast, they found that TAC levels were significantly lower in patients than in controls (19).

CCHF may be possibly related to increased free radical production and antioxidant depletion, and oxidative stress may be implicated in CCHF pathogenesis. Therefore, this study aimed to evaluate the functional integrity of the endothelium by determining TAC, TOS, and OSI values, as well as plasma total thiol (TTL) levels, as an essential component of antioxidant defense mechanisms, in pediatric and adult CCHF patients. The differences in oxidant mechanisms in relation to clinical variability of CCHFV infection were also investigated. To the best of our knowledge, this study is the first of its kind in the English literature.

MATERIALS AND METHODS

Patient selection: The study protocol was approved by the ethics committee of the Refik Saydam National Public Health Agency of the Ministry of Health as well as the local ethics committee of the Ankara University. Patients with acute febrile syndrome highly suggestive of CCHF (characterized by malaise, bleeding, leucopenia, and thrombocytopenia) who visited Cumhuriyet University and Hacettepe University hospitals between April 2010 and September 2011 from various regions of northeastern Anatolia and the southern Black Sea were approached for eligibility. Only CCHF patients confirmed by the virology laboratory of Refik Saydam National Hygiene Center using immunological specific enzyme-linked immunosorbent assay (ELISA) for IgM and molecular assays (reverse transcription [RT]-PCR, direct sequence analyses) were included in the study. Blood samples were obtained upon presentation, and stored at −80°C for measurement of the study parameters at the end of the study period. Informed consent was obtained from all patients and/or their guardians before investigations of the oxidative status. No additional samples were obtained for the purpose of this study. The control groups consisted of age- and gender-matched healthy individuals. Based on age and CCHF status, the patients were divided into 4 groups: group 1, children with CCHF; group 2, adults with CCHF; group 3, healthy children; and group 4, healthy adults.

TAC, TOS, and thiol measurement: Serum TAC levels were measured as described by Erel (20), which was based on the bleaching of the characteristic color of a more stable 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) radical cation by antioxidants. Results were expressed in mmol/L Trolox equivalents. The total oxidant status was measured as described by Erel (21), which was based on the oxidation of ferrous to ferric ions in the presence of various oxidative species in acidic medium and the measurement of the ferric ion concentration by xylenol orange. Results were expressed in μmol/L of H_2O_2. The TOS:TAC ratio was defined as the OSI and used as an indicator of the degree of oxidative stress (22). To calculate OSI, the unit for TAC (mmol/L Trolox equivalents) was converted to μmol/L Trolox equivalents, and the OSI value was calculated as OSI = TOS (μmol/L)/TAC (μmol/L Trolox equivalents/L) × 100.

Total serum thiol or sulfhydryl group concentrations were measured by the methods originally modified by Hu (23). Thiols interact with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and form a highly colored anion with a maximum peak at 412 nm (e412 = 13,600 M^{-1} cm^{-1}). In the manual protocol described by Hu (23), a 25-μL aliquot of fresh serum was mixed with 1 mL of Tris-EDTA buffer (0.25 mmol/L Tris base, 20 mmol/L EDTA, pH 8.2), and the absorbance at 412 nm was read. Next, a 25-μL aliquot of DTNB stock solution (10 mmol/L, in absolute methanol) was added to the solution. After 15 min at ambient temperature, the absorbance was read again together with a DTNB blank. The sulfhydryl group concentration was calculated using reduced glutathione as a sulfhydryl group standard, and the result was expressed in μmol/L.

Statistical analysis: Statistical analyses were performed using SPSS software for Windows ver.15.0 (SPSS Inc., Chicago, Ill., USA). Depending on the normality of distribution, values for continuous variables were provided either as means ± standard deviations or as medians (minimum–maximum). Frequencies of nominal variables were presented as percentages. For continuous variables with a normal distribution, more than 2 groups were compared using Welch univariate analysis of variance (ANOVA), whereas for abnormally distributed variables, the Kruskal–Wallis test was preferred. Multiple comparisons were made using the Games–Howell test or Mann–Whitney U-test with Bonferroni correction. For 2-group comparisons of independent variables, the Student's *t*-test was used as a

parametric test, whereas the Mann–Whitney U-test was the preferred non-parametric test. Cut-off values to distinguish between the study and control groups were determined using ROC analysis, after which sensitivity, specificity, and negative and positive predictive values (NPV and PPV, respectively) were calculated. A probability (*P*)-value of <0.05 was considered statistically significant.

RESULTS

Serum samples were collected from a total of 34 children (mean age, 11.95 ± 4.69 years; range, 0.5–18 years; female-to-male ratio, 0.61) and 41 adults (mean age, 42.78 ± 15.24 years; range, 19–71 years; female-to-male ratio, 1.05) who were diagnosed with CCHF. The control group consisted of age- and gender-matched 20 healthy children and 41 healthy adults.

There were no statistically significant differences in mean TAC values between the CCHF patients (groups 1 and 2) and healthy controls (groups 3 and 4) ($P > 0.05$). Similarly, no significant differences were observed following 2-group comparisons (group 1 vs. group 2, group 1 vs. group 3, and group 2 vs. group 4; $P > 0.05$ for all).

The mean TOS and OSI values were significantly lower in CCHF patients compared with healthy controls ($P < 0.001$ for both). A 2-way comparison of mean TOS and OSI values between children and adults with CCHF revealed no statistically significant differences. Although the mean OSI value was significantly lower in children with CCHF compared with their healthy counterparts ($P < 0.05$), a similar difference was not observed with TOS ($P > 0.05$). In adults, the mean TOS and OSI values in CCHF patients were significantly lower than in healthy controls ($P < 0.001$). The mean TTL value was significantly lower in CCHF patients compared with healthy controls, and a statistically significant difference persisted following 2-way comparisons of children (group 1 vs. group 3) and adults (group 2 vs. group 4) ($P < 0.05$ for both comparisons). The difference in mean TTL values between children and adults with CCHF was statistically insignificant. TAC, TOS, OSI, and TTL values, as well as results of the 2-way comparisons are summarized in Table 1.

The optimal cut-off values for TOS, OSI, and TTL were identified by plotting ROC curves. ROC curves of all CCHF patients versus healthy controls are depicted in Fig. 1. The area under the curve, specificity, sensitivity, PPV, NPV, and cut-off levels are listed in Table 2. The calculated cut-off values for TOS, OSI, and TTL to distinguish CCHF patients with were 5.02, 0.16, and 178.13, respectively, with respective specificities of 96.7%, 98.4%, and 83.6%. The PPVs for the 3 set cut-off values were 95.7%, 97.8%, and 87%, respectively, with respective sensitivities of 60.3%, 60.3%, and 97.1%. NPVs for these cut-off values were 67%, 67.4%, and 96.2%, respectively.

Although there were no fatalities among the children, 3 (7.3%) of the 41 adults with CCHF succumbed to the

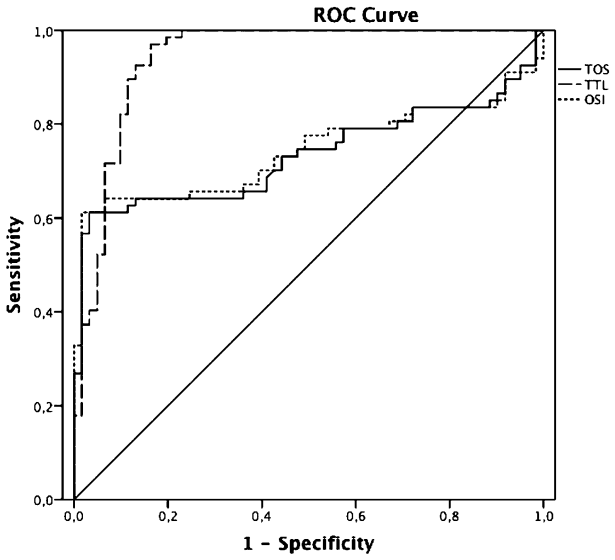

Fig. 1. ROC curves for all CCHF patients versus healthy controls; total oxidant status (black line), oxidative stress index (black dots), and plasma total thiol (interrupted line).

Table 1. TAC, TOS, OSI, and TTL values in patient and control groups

Parameter	Group 1 (children) $n = 34$	Group 2 (adults) $n = 41$	Group 3 (healthy children) $n = 20$	Group 4 (healthy adults) $n = 41$	*P*
TAC[1] (mmol Trolox equiv./L)	2.66 ± 0.31	2.66 ± 0.52	2.65 ± 0.68	2.73 ± 0.31	>0.05
TOS[2] (μmol H_2O_2/L)	7.27 (0.98–64.6)	2.37 (0.72–75.9)	10.38 (1.97–91.2)	12.03 (8.35–23.3)	>0.05*
					>0.05**
					<0.001***
OSI[2] (arbitrary unit)	0.28 (0.03–2.87)	0.09 (0.03–4)	0.51 (0.08–2.10)	0.46 (0.24–0.89)	>0.05*
					>0.05**
					<0.001***
TTL[1] (μmol/L)	128.40 ± 32.38	110.77 ± 41.50	167.86 ± 37.79	607.75 ± 62.10	>0.05*
					<0.05**
					<0.001***

TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index; TTL, plasma total thiol.
[1]: Values are given as mean ± standard deviation.
[2]: Values are given as median (minimum–maximum).
*Group 1 vs. Group 2.
**Group 1 vs. Group 3.
***Group 2 vs. Group 4.

Table 2. The area under the curve, cut-off, specifity, sensitivity, positive predictive and negative predictive values of TOS, OSI, and TTL

		AUC	Cut-off level	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
TOS	Group 1 + 2 vs. 3 + 4	0.718	5.02	96.7	60.3	95.7	67
OSI		0.725	0.16	98.4	60.3	97.8	67.4
TTL		0.942	178.1	83.6	97.1	87	96.2

TOS, total oxidant status; OSI, oxidative stress index; TTL, plasma total thiol; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

infection. Because of the low number of fatalities, we could not compare the oxidative status parameters between the surviving and deceased patients.

DISCUSSION

To date, little is known about the pathogenic mechanisms of CCHFV, and data in the literature are limited. CCHF is similar to other viral hemorrhagic fevers, i.e., it targets and impairs the function of cells that initiate antiviral immune responses, eventually leading to vascular dysregulation (4). Similarities in ROS-induced vascular changes and those observed in CCHF prompted us to investigate the potential role of ROS in the pathogenesis of this disease. To the best of our knowledge, the present study is the first in the English literature to investigate the role of pro- and antioxidant mechanisms in CCHF pathogenesis by evaluating TAC, TOS, OSI, and plasma TTL levels. Here, we observed significantly lower TOS and OSI values in CCHF patients compared with healthy age- and gender-matched controls, with no difference in TAC values. This finding is a paradox that is difficult to explain; however, one could argue that the timing of sampling or rather methodological limitations may have led to findings that were not revealed in our small study population. Our results suggested that CCHF-associated endothelial damage is not adequately explained solely by pro- and antioxidant mechanisms.

TTLs are extraordinarily efficient antioxidants with the ability to react with free radicals to protect against ROS-induced damage (24). Both intracellular and extracellular redox states of thiols play critical roles in the determination of protein structure and function, regulation of enzymatic activity of transcription factors, and promotion of antioxidant protection (25). TTL groups are found in all cells of body and are indispensable for cell survival. Proteins constitute the main antioxidant component of serum, and the sulfhydryl groups are primarily responsible for their antioxidative effects. TTL levels have also been shown to be positively correlated with thrombocyte counts and negatively correlated with the risk of bleeding. However, TTLs may increase bleeding tendency by detrimentally affecting the coagulation cascade (24,25). These findings were the incentive behind the evaluation of TTL levels in our study, which revealed significantly lower levels in CCHF patients compared with healthy controls. This finding could be attributable to the positive correlation between TTL levels and thrombocyte counts and negative correlation between TTL levels and risk of bleeding, which was frequently observed in CCHF patients. In contrast, high TTL levels can be protective against the development of CCHFV infections. It is possible that a reduction in the TTL levels is more important in CCHF pathogenesis than reductions in TAC and TOS.

There are few reports regarding the effects of the antioxidant NO, which can protect cells against oxidative injury, inhibit leukocyte adhesion, and participate in antimicrobial defense, in CCHF pathogenesis. It has been suggested that NO may hinder the early stages of viral replication, thereby preventing viral spread as well as promoting viral clearance and host recovery (26). Tutuncu et al. (27) observed significant increases in NO levels in CCHF patients. Other investigators have reported higher NO levels in patients with non-fatal CCHF compared with those who eventually died of CCHF, suggesting that NO may have a protective role against CCHFV infection. Based on our findings, we propose that higher serum TTL levels play a protective role in CCHFV infection.

CCHF is more fatal in adults than in children in whom the mortality rates tend to be low. Although numerous studies have attempted to determine predictors of CCHF severity and mortality in adults (28–30), similar studies have not been conducted in children. The reasons behind the more severe course in adults compared with children remain unknown. In the present study, a comparison between children and adults did not reveal significant differences in TAC, TOS, OSI, and TTL values. The milder clinical course of CCHF in children could not be explained by these parameters alone; therefore, further multicenter studies are warranted to help elucidate the varying clinical course of CCHFV infection in children and adults.

The clinical picture of CCHF is most likely a result of a complex interplay between direct and indirect effects of viral infection of the endothelium. Our study is the first to evaluate TAC, TOS, OSI, and TTL for their potential role in CCHF pathogenesis. Although we could not demonstrate significant changes in TAC levels between CCHF patients and healthy controls, the mean TOS and OSI levels were significantly lower in the former. We also observed significantly lower TTL levels in CCHF patients. According to our results, TTL levels may be a useful predictor to diagnose or follow-up CCHF patients. Although further studies including serial patient samples are obviously needed to help elucidate the role of pro- and antioxidant pathways in CCHF pathogenesis, our findings undoubtedly suggest that other undetermined factors are likely also involved.

Conflict of interest None to declare.

REFERENCES

1. Hoogstraal, H. (1979): The epidemiology of tick borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J. Med. Entomol., 15, 307–417.
2. Ergonul, O., Celikbas, A., Dokuzoguz, B., et al. (2004): The characteristics of Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and the impact of oral ribavirin therapy. Clin. Infect. Dis., 39, 285–289.
3. Whitehouse, C.A. (2004): Crimean-Congo hemorrhagic fever. Antiviral Res., 64, 145–160.
4. Ergonul, O. (2006): Crimean-Congo haemorrhagic fever. Lancet Infect. Dis., 6, 203–214.
5. Tezer, H., Sucakli, I.A., Sayli, T.R., et al. (2010): Crimean-Congo hemorrhagic fever in children. J. Clin. Virol., 48, 184–186.
6. Connolly-Andersen, A.M., Moll, G., Andersson, C., et al. (2011): Crimean-Congo hemorrhagic fever virus activates endothelial cells. J. Virol., 85, 7766–7774.
7. Bodur, H., Akinci, E., Ongürü, P., et al. (2010): Evidence of vascular endothelial damage in Crimean-Congo hemorrhagic fever. Int. J. Infect. Dis., 14, 704–707.
8. Ozturk, B., Kuscu, F., Tutuncu, E., et al. (2010): Evaluation of the association of serum levels of hyaluronic acid, sICAM-1, sVCAM-1, and VEGF-A with mortality and prognosis in patients with Crimean-Congo hemorrhagic fever. J. Clin. Virol., 47, 115–119.
9. Krötz, F., Sohn, H.Y. and Pohl, U. (2004): Reactive oxygen species: players in the platelet game. Arterioscler Thromb. Vasc. Biol., 24, 1988–1996.
10. Levi, M., van der Poll, T. and Büller, H.R. (2004): Bidirectional relation between inflammation and coagulation. Circulation, 109, 2698–2704.
11. Herkert, O., Djordjevic, T., BelAiba, R.S., et al. (2004): Insights into the redox control of blood coagulation: role of vascular NADPH oxidase-derived reactive oxygen species in the thrombogenic cycle. Antioxid. Redox Signal., 6, 765–776.
12. Weber, F. and Mirazimi, A. (2008): Interferon and cytokine responses to Crimean-Congo hemorrhagic fever virus: an emerging and neglected viral zonoosis. Cytokine Growth Factor Rev., 19, 395–404.
13. Sonmez, M., Aydin, K., Durmus, A., et al. (2007): Plasma activity of thrombin activatable fibrinolysis inhibitor in Crimean-Congo hemorrhagic fever. J. Infect., 55, 184–187.
14. Nowak, P., Olas, B. and Wachowicz, B. (2010): Oxidative stress in haemostasis. Postepy. Biochem., 3, 239–247.
15. Gantt, K.R., Goldman, T.L., McCormick, M.L., et al. (2001): Oxidative response of human and murine macrophages during phagocytosis of *Leishmania chagasi*. J. Immunol., 167, 893–901.
16. Murray, H.W. and Teitelbaum, R.F. (1992): L-arginine-dependent reactive nitrogen intermediates and the antimicrobial effect of activated human mononuclear phagocytes. J. Infect. Dis., 165, 513–517.
17. Serefhanoglu, K., Taskin, A., Turan, H., et al. (2009): Evaluation of oxidative status in patients with brucellosis. Braz. J. Infect. Dis., 13, 249–251.
18. Karaagac, L., Koruk, S.T., Koruk, I., et al. (2011): Decreasing oxidative stress in response to treatment in patients with brucellosis: could it be used to monitor treatment? Int. J. Infect. Dis., 15, 346–349.
19. Karsen, H., Sunnetcioglu, M., Ceylan, R.M., et al. (2011): Evaluation of oxidative status in patients with Fasciola hepatica infection. Afr. Health Sci., 11, 14–18.
20. Erel, O. (2004): A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radicalcation. Clin. Biochem., 37, 277–285.
21. Erel, O. (2005): A new automated colorimetric method for measuring total oxidant status. Clin. Biochem., 38, 1103–1111.
22. Harma, M., Harma, M. and Erel, O. (2005): Measurement of the total antioxidant response in preeclampsia with a novel automated method. Eur. J. Obstet. Gynecol. Reprod. Biol., 118, 47–51.
23. Hu, M.L. (1994): Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol., 233, 380–385.
24. Sen, C.K. (1998): Redox signaling and the emerging therapeutic potential of thiol antioxidants. Biochem. Pharmacol., 55, 1747–1758.
25. Wlodek, L. (2002): Beneficial and harmful effects of thiols. Pol. J. Pharmacol., 54, 215–223.
26. Reiss, C.S. and Komatsu, T. (1998): Does nitric oxide play a critical role in viral infections? J. Virol., 72, 4547–4551.
27. Tutuncu, E.E., Gurbuz, Y., Ozturk, B., et al. (2010): Serum nitric oxide levels in patients with Crimean-Congo haemorrhagic fever. Scand. J. Infect. Dis., 42, 385–388.
28. Cevik, M.A., Erbay, A., Bodur, H., et al. (2008): Clinical and laboratory features of Crimean-Congo hemorrhagic fever: predictors of fatality. Int. J. Infect. Dis., 12, 374–379.
29. Ergonul, O., Celikbas, A., Baykam, N., et al. (2006): Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. Clin. Microbiol. Infect., 12, 551–554.
30. Swanepoel, R., Gill, D.E., Shepherd, A.J., et al. (1989): The clinical pathology of Crimean-Congo hemorrhagic fever. Rev. Infect. Dis., 11, 794–800.