

Oxidative stress in the adult and pediatric patients with Crimean-Congo haemorrhagic fever

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ABSTRACT

Background & objectives: Crimean-Congo haemorrhagic fever (CCHF) can be fatal with bleeding, shock and disseminated intravascular coagulopathy (DIC). Although similar genetic strains have been defined, the causes of the clinical differences between the cases are yet to be found. We aimed to demonstrate the balance between oxidant and antioxidant system in CCHF.

Methods: In this study, the patient group consisted of 72 cases with a positive diagnosis of CCHF according to PCR/ELISA outcome among the patients referred to Cumhuriyet University, Medical Faculty in 2010. A total of 74 volunteers who were not having any viral or metabolic disease, non-smokers and age and sex matched with the patients group were enrolled as the control group. Both in the controls and the patients, individuals aged under 16 yr were defined as group 1 and the individuals aged over 16 yr as group 2. The serum samples were stored at -80°C until the study was carried out. All the samples were simultaneously thawed. In these cases, total antioxidant capacity (TAC), total oxidative status (TOS), oxidative stress index (OSI), lipid peroxide (LPO), paraoxonase (PON) and arylesterase were analyzed with the ELISA method. OSI was calculated.

Results: Levels of TOS, OSI and LPO were found significantly higher in CCHF patients in both the groups ($p < 0.05$), whereas levels of TAC, PON1 and arylesterase were lower in CCHF patients compared to the controls, but low level of TAC in the group 1 was not statistically significant.

Interpretation & conclusion: Our study demonstrated increased oxidative stress in CCHF patients in both groups 1 and 2. In order to prevent tissue damage which might be developed due to the oxidative stress in CCHF patients, further comprehensive studies should be conducted to define whether the adding antioxidants to the treatment would be helpful or not.

Key words Arylesterase; Crimean-Congo haemorrhagic fever; oxidative stress; paraoxonase

INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) is a viral haemorrhagic disease caused by Nairovirus from Bunyavirus family and proceeds with high fever and diffuse bleeding. The disease transmits by tick bite, tick removal and by contact with the cases in the acute period of infection and with blood or tissues of viremic animal. The disease which becomes increasingly widespread has been identified in the middle east, western Europe, Asia and Africa continents. In Turkey, an epidemic of uncertain cause drew attention for the first time especially in Tokat, and in Amasya and Sivas provinces with history of tick contact, progressed with fever and bleeding in the spring and summer months of 2002. The disease was understood to be CCHF in 2003¹⁻². The epidemic which has begun in 2002 is currently being continued and according to the records of the Ministry of

Health, proven number of CCHF cases reached to 4453 as of 2009³.

Infected mononuclear phagocytic cells, liver and endothelial cells are known to play a crucial role in pathogenesis of CCHF⁴. The disease courses subclinical in some cases, while it may result in death with the development of shock and disseminated intravascular coagulopathy (DIC). Although similar genetic strains have been defined, the causes of the clinical differences between the cases are yet to be investigated.

Reactive oxygen species (ROS) occur in organisms depending on several mechanisms. Energy metabolism in the mitochondria, in active phagocytic leukocytes causes production of superoxide, peroxide, hydroxyl and other free radicals that are known as ROS and derived from oxygen in hundreds of biological aerobic molecular environment such as various enzymes (xanthine oxidase, tryptophan dioxygenase, NADPH oxidase, etc.), hydro-

quinone, flavins, thiols, catecholamines, ferredoxin and reduced nucleotides⁵⁻⁶. All the biomolecules (protein, lipid, nucleic acids and the carbohydrates) are affected by free radicals. When production of free radicals (in the cases of oxidative stress conditions) exceeds antioxidant defense system, permanent tissue damage and chronic diseases develop⁷. Reactive oxygen species have been shown to play an important role in the pathogenesis of rheumatoid arthritis, atherosclerosis, acute and chronic inflammatory diseases⁸. In CCHF patients, viral infection, high fever and increased breakdown products are likely to clean the oxidation paths and resultant increased oxidative stress may trigger the prognosis of the disease. If oxidative stress exceeds the antioxidant capacity or if oxidative stress remains unchanged and antioxidant capacity decreases, tissue damage develops due to oxidative damage.

Increased ROS react with the particularly double bonds of multi unsaturated fatty acid, resulting in occurrence of malondialdehyde (MDA) compounds those are the final products of lipid peroxides⁹. Recent studies have shown that paraoxonase (PON1) has an antioxidant effect against lipid peroxidation caused by free radicals on cell membranes and lipoproteins¹⁰. Paraoxonase multi-gene family located in q21-22 region of the 7th chromosome in human consists of three members called PON1, PON2 and PON3¹¹. PON1, is a calcium dependent ester hydrolase both having paraoxonase and arylesterase activity. This is a protein mainly synthesized in the liver and has hydrolytic effect on several substrates, including serum paraoxonase enzyme-1, organophosphates, arylesterases and lactones that are released to serum circulation¹². Primary physiologic role of PON1 is not fully understood, although recent studies reported it to be associated with the HDL cholesterol and to play a protective role against the oxidative modification of LDL cholesterol, preventing lipid peroxidation and to have antioxidant and anti-inflammatory characteristics¹³. Furthermore, in the patients who suffer from the diseases characterized by oxidative damage, HDL was found to be more prone to peroxidation and correlated with the decreased activity of PON1¹⁴.

In this study, we aimed to demonstrate the balance between oxidant and antioxidant systems by measurement of total antioxidant capacity (TAC), total oxidative status (TOS), oxidative stress index (OSI), lipid peroxide (LPO), paraoxonase (PON) and arylesterase levels in order to get knowledge about the antioxidant system in CCHF patients.

MATERIAL & METHODS

In this study, serum samples were taken from the

patients referred to Cumhuriyet University Medical Faculty, Department of Emergency Medicine, Department of Infectious Diseases and Department of Pediatrics with a history of tick bite in 2010. Out of these samples, 72 cases with a positive diagnosis of CCHF according to the PCR/ELISA results from the Refik Saydam Hygiene Center of Ankara, Turkey were selected for the study. As the control group; blood specimens were collected from age, and sex matched 30 persons aged <16 yr and 44 persons aged >16 yr old, those without history of viral or metabolic disorder, and are non-smokers. Both in the controls and the patients, individuals aged <16 yr were defined as group 1 and the individuals aged >16 yr as group 2. The serum samples of both groups were centrifuged at 4000 rpm for 5 min using Hettich Universal 30 centrifuge device. The serum samples were stored at -80°C until use. All the samples were simultaneously dissolved or thawed. In these cases, TAC, TOS, PON and arylesterase were analyzed in *Synchron LX 20* autoanalyzer using *Rel Assay Diagnostic* brand kits and Cayman LPO was analyzed in the assay kit "Grifols" brand "Triturus" model ELISA device. OSI was calculated.

Total antioxidant capacity (TAC)

TAC levels were measured using commercially available kits (Relassay, Turkey). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS [2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation by antioxidants. The assay has excellent precision values, which are <3%. The results were expressed as mmol Trolox equivalent/l¹⁵.

Total oxidant status (TOS)

TOS levels were measured using commercially available kits (Relassay, Turkey). In this new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The colour intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ equivalent/l)¹⁶.

Oxidative stress index (OSI)

The ratio of TOS to TAC was accepted as the OSI. For calculating, the resulting unit of TAC was converted

to $\mu\text{mol/l}$, and the OSI value was calculated according to the following formula¹⁷⁻¹⁹:

$$\text{OSI (Arbitrary unit)} = \frac{\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/l})}{\text{TAC } (\mu\text{mol Trolox equivalent/l})}$$

Paraoxonase and arylesterase

Paraoxonase and arylesterase activities were measured by using commercially available kits (Relassay, Turkey). The rate of paraoxon hydrolysis (diethyl p-nitrophenyl phosphate) was measured by monitoring the increase of absorption at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorption coefficient at pH 8.5, which was 18.290 $\text{M}^{-1} \text{cm}^{-1}$. Paraoxonase activity was expressed as U/L serum. Phenyl acetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorption coefficient of the produced phenol, 1310 $\text{M}^{-1} \text{cm}^{-1}$. One unit of arylesterase activity was defined as 1 μmol phenol generated per minute under the above conditions and expressed as U/L.

Statistical analysis

Data from the study were loaded to SPSS 15 software. Analysis of the data was carried out using Chi-square, independent two-sample *t*-test and correlation test. The data were expressed as numbers and the level of significance was considered as 0.05.

This study was conducted with the approval of the Cumhuriyet University Medical Faculty Ethics Committee dated 9/12/2009 and B.30.2.CUM.0.1H.00.00/27 numbered decision for compliance.

RESULTS

Demographic features of the patients included in the

study are shown in Table 1. Out of the control group aged <16 yr, 23 (76.7%) were males and 7 (23.3%) were females with a mean age of 10.2 ± 4.44 yr. Of the patients group, 22 (78.6%) of 28 persons were males and 6 (21.4%) were female patients with a mean age of 10.14 ± 4.03 yr (Table 1). Of the group aged >16 yr, 27 (61.4%) of 44 persons were males and 17 (38.6%) were females with a mean age of 45.22 ± 16.33 yr. Of the patients group, 28 (63.6%) of 44 persons were males and 16 (36.4%) were female patients with a mean age of 52.11 ± 17.83 yr. On comparison, no significant difference was found between the controls and patients in terms of age and gender ($p > 0.05$).

Levels of TOS, OSI and LPO were found significantly higher in CCHF patients in the groups 1 and 2 compared to the controls ($p < 0.05$) (Table 1). Despite levels of TAC, PON1 and arylesterase were lower in CCHF positive cases in the groups 1 and 2 compared to the controls, low level of TAC was not statistically significant (Table 1).

DISCUSSION

Redox balance is a crucial factor for numerous cellular functions. This balance provides important contribution to the pathophysiology of several diseases, including viral infections. Virus induced oxidative stress may mediate to release of proinflammatory cytokines. In some cases, inflammatory response of the host may contribute to the pathophysiology of the disease²⁰. Levels of antioxidant enzymes are sensitive to oxidative stress. ROS's increase or decrease depends on the disease. In this study, levels of TOS, OSI and LPO were found significantly increased in the patients in both the groups compared to the controls ($p < 0.05$). Despite levels of TAC, PON1 and arylesterase were lower in CCHF positive cases in the groups 1 and 2 compared to the controls, low level of

Table 1. Demographic features and comparison of the patients and controls in terms of the measured parameters

Features	Group 1 (<16 yr)		<i>p</i> -value	Group 2 (>16 yr)		<i>p</i> -value
	Control n = 30	CCHF patients n = 28		Control n = 44	CCHF patients n = 44	
Age	10.20 \pm 4.44	10.14 \pm 4.03		45.22 \pm 16.33	52.11 \pm 17.83	
Gender						
Female	7 (23.3)	6 (21.4)		17 (38.6)	16 (36.4)	
Male	23 (76.7)	22 (78.6)		27 (61.4)	28 (63.6)	
TOS ($\mu\text{mol H}_2\text{O}_2$ equiv/l)	3.98 \pm 1.68	6.47 \pm 2.43	0	6.49 \pm 2.42	9.32 \pm 3.09	0
TAC ($\mu\text{mol trolox equiv/l}$)	1.25 \pm 0.31	1.23 \pm 0.21	0.737	1.22 \pm 0.25	0.94 \pm 0.29	0
OSI	0.32 \pm 0.13	0.53 \pm 0.20	0	0.55 \pm 0.21	1.07 \pm 0.43	0
LPO ($\mu\text{mol/l}$)	1.52 \pm 0.62	2.51 \pm 0.55	0	1.34 \pm 0.68	2.46 \pm 1.14	0
PON1 (U/L)	80.18 \pm 19.35	55.20 \pm 24.60	0	86.36 \pm 24.40	57.2 \pm 14.08	0
Arylesterase (U/L)	23,968 \pm 506	22,126 \pm 2065	0	16,205 \pm 2840	14,127 \pm 1771	0

Figures in parentheses indicate percentages.

TAC was not statistically significant. These results suggested that oxidative stress had increased, and the anti-oxidant system decreased in CCHF patients related to the viral infection.

In our previous study, we showed increase in the level of SOD (superoxide dismutase) no change in the levels of CAT (catalase), GSHP (glutathione peroxidase) and GR (glutathione reductase) decrease in the level of vitamin E and increase in the levels of plasma and erythrocyte MDA (malondi-aldehyde) in CCHF patients²¹.

Güner *et al*²² showed that the level of TOS significantly increased in CCHF patients compared to the controls. This finding supports our study. Gil *et al*²³ demonstrated oxidative stress to be increased in the patients with dengue fever, which is another viral haemorrhagic fever, compared to the controls. Akaike *et al*²⁴ showed that the levels of superoxide anion and hydrogen peroxides increase in viral infections. All these studies indicate that the oxidative stress increases in relation to viral infection, and this result is consistent with our findings.

Increased oxidative stress may lead to the hepatic cellular damage²⁵. Several studies demonstrated increased levels of AST, ALT, ALP, GGT and LDH in CCHF patients²⁶. Oxidative stress is likely to provide contribution to the increased levels of AST, ALT, ALP, GGT and LDH in CCHF patients. Halliwell *et al*²⁷, reported lipid peroxidation might be increased due to the inflammatory response in viral infections and increased level of anti-oxidant was the earliest indicator of decreased oxidative stress during the healing process. LPO and ROS were shown to cause fibrosis in the liver tissue in viral infections with insufficient antioxidant capacity^{28–29}.

In a study, it was reported that PON1 might reduce lipid peroxides such as oxidated polyunsaturated fatty acids and H₂O₂³⁰. Under oxidative stress, lipid peroxidation occurs in the lipids found not only in LDL, but also in HDL³¹. PON1 was reported to protect both the LDL and HDL against oxidation³⁰. PON1 is proposed to provide contribution to antioxidant impact through HDL and might be associated with the metal ion chelation and/or peroxidase like activity under the inhibitor effect of HDL. HDL-PON1 long chain oxidized phospholipids have the ability to hydrolyze³². We found decreased levels of PON1/arylesterase in CCHF patients both in groups 1 and 2 compared to the controls ($p < 0.05$). Low levels of PON1/arylesterase in CCHF patients may be due to various factors; liver is known to be affected in hepatic patients. PON1/arylesterase is synthesized in the liver³³ and low level of PON1/arylesterase in CCHF patients may be due to the expression. Recent studies reported an opposite correlation between increased ROS

and paraoxonase-1/arylesterase which is an anti-oxidant enzyme and, levels of PON1/arylesterase decreased when ROSs increased^{35–36}. Low levels of PON1/arylesterase may be due to increased oxidative stress.

In an *in vitro* study by Kumon *et al*³⁶, an opposite correlation was reported between PON1 and IL-1 and TNF- α ; increased IL-1 and TNF- α led to decrease in serum paraoxonase activity. In the studies on CCHF patients, levels of TNF- α , IL-1, and IL-6 were demonstrated to increase³⁷. Low levels of PON1/arylesterase may be due to increased TNF- α and IL.

Tarçın *et al*³⁸ stated that the most important factor in paraoxonase-1/arylesterase activity was age; and the enzyme activity, which was maximum in the infants and gradually decreased with the age³⁸. In our study, levels of paraoxonase-1/arylesterase were found to be lower in the persons aged <16 yr than in those >16 yr old ($p > 0.05$). Our finding was not at all parallel to that in the study by Tarçın *et al*³⁸. Low levels of paraoxonase-1/arylesterase in the children compared to the adults in our study group might be attributed to the limited number of the cases.

In conclusion, oxidative stress was found to be increased in CCHF patients both in the groups 1 and 2. Increased oxidative stress has been emphasized to cause damage in many tissues. There are no studies on this subject in CCHF patients. In order to prevent tissue damage that might be developed due to the oxidative stress in CCHF patients, further comprehensive studies should be conducted to define whether adding antioxidants to the treatment would be helpful or not.

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