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Full Length Research Paper

Malondialdehyde, vitamin E, and anti-oxidant enzyme activity levels in patients with crimean-congo hemorrhagic fever

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Crimean-congo hemorrhagic fever (CCHF) is a viral disease that can be seen in people epidemically or as single patient. CCHF is a highly deadly disease caused by Nairovirus from Bunyaviridae family. Endothelial damage caused by secreted cytokines plays an important role in the pathophysiology of the disease. Reactive oxygen species (ROS) are highly cytotoxic. Intracellular and extracellular antioxidants are the mechanisms that protect the cell from the cytotoxic effects of the ROS. In this study, malondialdehite (MDA), superoxide dismutase (SOD), glutation peroxidase (GSHPx), glutation reductase (GR), catalase (CAT) enzyme activities and vitamin E – one of the antioxidant vitamins, values are examined to evaluate how oxidant/antioxidant value is affected in patients with CCHF. 47 patients diagnosed as CCHF with control group that is made of 41 healthy individuals that matched to patient group as sex and age contributed to study. All individuals' plasma SOD, CAT, GSHPx, GR, and vitamin E values and erythrocyte MDA levels were determined. SOD enzyme activity is increased ($p<0.05$) and CAT, GSHPx, GR enzyme activities did not change in CCHF patient ($p>0.05$). Vitamin E values turned out to be lower than control group ($p<0.05$). Plasma and erythrocyte MDA levels were higher than control group ($p<0.05$).

Key words: Crimean-congo hemorrhagic fever, antioxidants, vitamin E, malondialdehyde.

INTRODUCTION

CCHF is transmitted to people from Hyalomma type ticks. It has a high mortality ratio and characterized with hemorrhage and fever, thrombocytopenia, leucopenia, increase in transaminases, and abnormal homeostasis tests. Its cause is a RNA virus called CCHF virus from *Nairovirus* sp. which belongs to Bunyaviridae family. CCHF virus transmitted to people with tick bites or tick contact, contact with blood, body secretion or body fluids and other infectious tissue of infected people or animals. It is reported for the first time in May, June, and July of 2002 in Tokat, Amasya, and Sivas. In 2003, with the new reports of a similar disease with a similar prognosis, it is understood that disease was KKHF. 133 subjects were reported in 2003 and 6 of the subjects died. 13 patients out of 249 in 2004, 13 patients out of 266 in 2005, 27 patients out of 438 in 2006, 33 patients out of 717 were dead. In 2008, the number increased to 63 patients out of 1315 subjects. Prevalence of the disease increased since

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Abbreviations: SOD, superoxide dismutase; **CAT,** catalase; **GSH-Px,** glutation peroxidase; **GR,** glutation reductase; **MDA_{eryt}**, erythrocyte malondialdehite; **MDA_{plas}**, plasma malondialdehite ; **E-vit,** vitamin E.

its first occurrence in our country. Death ratios vary between the reports but in our country it is determined as 5-6% (The Ministry of Health of Turkey).

Endothelial damage caused by cytokines plays an important role in the pathophysiology of the disease; however the complete pathogenesis is not fully understood. Exact diagnosis of the disease is done by PCR, indication of the genetic material or determination of specific IgM antibodies by ELISA technique. Main point of the treatment is supporting therapy. In treatment, the role of ribavirin despite its effect of decreasing viral replication, is controversial (World Health Organization, 2003; Mardani et al., 2003). Supporting therapy is the main therapy and it is important to maintain liquid- electrolyte balance and to transfuse blood, thrombocyte, and fresh frozen plasma in need. Today there is no approved vaccine for CCHF.

Reactive Oxygen Species (ROS) are highly toxic for the organism. ROS and free radicals play important role in many of the physiologic and pathologic processes. Organism is being protected from hazardous effects of endogen ROS by enzymatic and non-enzymatic anti-oxidant enzyme systems. ROS is being naturalized mainly by anti-oxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutation peroxidase (GSHPx) (Halliwell, 1994). In healthy individuals, oxidant levels are stabilized by anti-oxidants. Plasma and tissue anti-oxidants are enzymes such as transferrin, lactoferrin, SOD, CAT, GSHPx or small molecules such as vitamins E and C. These anti-oxidant molecules prevent the damage made by free radicals to the target biomolecules such as lipids, proteins, nucleic acids (Yazici and Köse, 2004).

SOD plays an important role in protection of cells against oxygen toxicity (Stefan et al., 2007; Crapo et al., 1992). CAT catalyses the transformation of H_20_2 to water and oxygen and protects the cell against the respiration explosions (Chelikani et al., 2005; Çimen et al., 2005). GSH-Px protects erythrocytes against oxidative damage, and catalyses degradation of some kind of hydrogen-peroxides (Jose et al., 2000). tocoferol -the most active form of Vitamin E- is a powerful anti-oxidant and protects polyunsaturated fatty acids found in cell membrane phospholipids from the effects of free radicals (Regina and Maret, 1999).

Even free radical reactions are required for defense mechanism of immune system cells such as neutrophils and macrophages, when exceeded in production they cause cell death and tissue damage. Balance between free radicals and defense system is important during an illness.

The most effected molecules from free radicals are biomolecule lipids. Some ROS causes lipid peroxidation in cytoplasm, mitochondria, cell nucleus and endoplasmic reticulum membranes. As a result of lipid peroxidation, membrane permeability increases and cell damage occurs. Membrane damage caused by lipid peroxidation is irreversible (Kilinç and Kilinç, 2002; Niki et al., 2005; from the free radicals. 3D structure of albumin and IgG can be destroyed. Cell and mitochondria DNA can be oxidized and this may cause damage in cellular life (Smith et al., 2004).

As the half -time of ROS is short, it is difficult to determine the concentrations in the body. Because of this, by determination of MDA -bio-marker of lipid peroxidation-, enzymes such as SOD, CAT, GSH-Px, and glutation reductase and -tocoferol (vitamin E) from anti-oxidant vitamins levels, indirect information can be obtained about free radicals.

In this study, we aimed to determine MDA level -occurred after lipid peroxidation-, enzyme activity levels of anti-oxidant enzymes such as SOD, GSH-PX, GR, CAT and vitamin E level of anti-oxidant vitamins to evaluate how oxidant/anti-oxidant balance is being affected in CCHF subjects.

METHODS

47 patients contributed to this study who went under surveillance in Cumhuriyet University Research and Education Hospital Infectious Diseases Department with CCHF prediagnosis and diagnosed as CCHF after tests were done in Refik Saydam Hıfzı Sıhha Institute. 41 people were chosen as control group who have similar traits such as age and sex with patient group.

Preparation of samples

Plasma and erythrocyte is taken and put into tubes that contain EDTA and heparin, from patients who diagnosed CCHF to measure GSH-PX, GR, SOD, CAT, MDA and vitamin E levels.

Acquisition of erythrocytes and plasma with EDTA

Blood samples taken into tubes that contain EDTA were centrifuged for 10 min at 1000 rpm at +4°C. Plasma part which is on top is taken away by aspiration without damaging leukocyte layer. 2 ml serum physiologic was added to erythrocyte suspension and mixed slowly and centrifuged for 10 min at 1000 rpm. After repeating this process 4 times, erythrocyte suspension that is obtained was saved at -80°C.

Determination of superoxide dismutase activity

Superoxide Dismutase Assay kit manufactured by Cayman chemical, measured by using 2100 C model ELISA reader manufactured by Rayto. 1 U SOD is identified as enzyme amount needed to naturalize 50% of superoxide radicals. In each erythrocyte suspension, hemoglobin is determined and SOD levels were calculated as U per g hemoglobin.

Determination of catalase activity

Catalase Assay Kit manufactured by Cayman chemical, using 2100 C model ELISA reader manufactured by Rayto. In each erythrocyte suspension, hemoglobin is determined and CAT levels are calculated as U per g hemoglobin.

Determination of glutation peroxidase activity

Northwest life science specialties, Glutathione Peroxidase Assay kit manufactured by LLC Company was used.. In each erythrocyte suspension, hemoglobin is determined and GR levels were calculated as U per g hemoglobin.

Determination of vitamin E

Vitamin E determination is done by the method based on the staining of filtrate obtained by absolute ethanol and xylene extraction, with $FeCL_3$ or 2,4,6-tripidil-s-triazin.

Determination of lipid peroxidation

Basic principles of the method based on the fact that a colorful complex with maximum absorbance at 532 nm wavelength occurs by the reaction of MDA -which is a result of lipid peroxdiation- and TBA (tiobarbutic acid).

Statistical analysis

Statistical evaluation of individuals in patient and control group is done with SPSS 14.0, significance test between two means in independent groups is done by using Man Whitney U test and Kruskal Waallis test. Results are mentioned as arithmetical mean ± SD and p is 0.05.

RESULTS

Mean age of patient group was 42.53 ± 20.38 years; mean age of control group was 42.63 ± 20.56 years. There was no significant statistical difference between groups in point of age ($p>0.05$). There was no statistical significant difference between groups in terms of sex. Between control and patient groups was not statistically significant difference in terms of age stratification ($p>0.05$) (Table 1).

When all subjects in control and patient groups was compared for enzyme activities, for CAT, GSH-Px and GR, the difference between the two groups was not statistically significant ($p>0.05$) but when SOD enzyme activity and MDA_{eryt}, MDA_{plas} E vitamin value was compared, the difference between the two groups was statistically significant (Table 2).

All age strata of control and patient groups was not statistically significant difference in terms of measured parameters ($p>0.05$). GR enzyme activity was higher in female individuals of control group ($p<0.05$) (Table 3). Between male and female individuals in the patient group was not difference in terms of measured parameters ($p>0.05$) (Table 4).

When measured parameters of male patients in both control and patient group was compared (Table 5) for CAT and GSH-Px, difference between the two groups was not statistically significant ($p>0.05$). SOD, GR, MDA_{plas} ve MDA_{eryt} levels were higher in patient group and vitamin E values were higher in patient group and these differences were statistically significant ($p<0.05$).

Table 1. Age stratification of control and patient groups.

Age	Control	Patient		Total	
		n	%	n	%
14	4	9.8	6	12.8	10
15-19	4	9.8	3	6.4	7
20-29	5	12.2	4	8.5	9
30-39	6	14.6	4	8.5	10
40-49	4	9.8	9	15.1	13
50-59	8	19.5	10	21.3	18
60	10	24.4	11	23.4	21
Total	41	100	47	100	21

$X^2= 2.85$; $p= 0.827$

When measured parameters of women both in control and patient group were compared, there were no difference between CAT, GSH-Px ve GR levels in both groups ($p<0.05$). SOD, MDA_{plas}, MDA_{eryt} values were higher in women who are in patient group and Vitamin E values were higher in women who are in control group and these differences were statistically significant (Table 6).

DISCUSSION

Inactivation role of antioxidants of virus activity has begun been understood in early 1970's, however, their metabolic role in viral infections was understood in later years (Peterhans, 1997). Oxidants take place in intracellular signaling and immune mechanisms as defense system. However, even high amount of ROS are not the primary reason for many of the diseases, they play role in the pathogenesis of diseases such as cancer, ischemia, immune deficiency, endocrine dysfunction and cause tissue damage. During the progress of the diseases when ROS levels increase antioxidant enzyme levels increase as response (Peterhans, 1997; Droge, 2002; Andrese et al., 2006).

Until now, no study has been done about oxidative stress and oxidant/anti-oxidant system evaluation of CCHF patients in the existing literature. In our study, based on the truth that no reliable data can be obtained just for evaluation of a single anti oxidant level, plasma vitamin E value and SOD, CAT, GSH-Px enzyme activity in erythrocytes were evaluated as well.

Oxidant and antioxidants show level differences in patients. While ROS tend to increase, antioxidants can change in both ways. Oxidant and antioxidant values and antioxidant activities evaluated in diseases such as depression, coronary artery disease, hypertension, hypotiroidism, Parkinson disease, allergic diseases, lung cancers and Alzheimer (Dominguez-Rodriguez et al., 2008; Bilici et al., 2001; Redón et al., 2003; Yilmaz et al.,

Table 2. Laboratory data of control and patient groups.

	Control (n = 41) $\overline{X}$ ± S	Patient (n= 47) $\overline{X}$ ±S	p value
SOD (U/gHb)	1199.52 ± 216.81	1798.85 ± 257.98	t=-11.700 p< 0.001
CAT (U/gHb)	12046.32± 1907.93	12754.15 ± 2129.11	t=-1.632 p= 0.106
GSH-Px (U/gHb)	19.226 ± 3.934	18.782 ± 2.877	t=0.610 p= 0.544
GR (U/gHb)	8.872 ± 1.399	9.342 ±1.275	t=-1.621 p= 0.109
$MDA_{eryt.}$ (µmol/gHb)	0.210 ± 0.048	0.290 ± 0.048	t=-7.570 p< 0.001
$MDA_{plas.}$ (µmol/L)	0.457 ± 0.164	0.784 ± 0.196	t=-8.193 p< 0.001
E-vit. (mg/dl)	1.092 ± 0.233	0.931 ± 0.122	t=4.130 p< 0.001

Table 3. Comparison of male and female individuals for measured parameters in control group.

	Male (n =16) $\overline{X}$ ± S	Female (n= 25) $\overline{X}$ ± S	p value
SOD (U/gHb)	1184.50 ± 224.82	1209.12 ± 215.66	p= 0.789
CAT (U/gHb)	11385.13 ± 1601.85	12469.48 ± 1994.31	p= 0.078
GSH-Px (U/gHb)	18.43 ± 3.68	19.73 ± 4.07	p= 0.285
GR (U/gHb)	8.28± 1.29	9.25± 1.35	p= 0.013
$MDA_{eryt.}$ (µmol/gHb)	0.203± 0.048	0.212± 0.050	p= 0.584
$MDA_{plas.}$ (µmol/L)	0.423± 0.162	0.478± 0.165	p= 0.261
E-vit. (mg/dl)	1.073± 0.145	1.105± 0.278	p= 0.415

Table 4. Comparison of male and female individuals for measured parameters in patient group.

	Male (n = 27) $\overline{X}$ ± S	Female (n = 20) $\overline{X}$ ± S	p value
SOD (U/gHb)	1762.21 ± 276.97	1848.32 ± 227.32	p= 0.302
CAT (U/gHb)	12382.52 ± 2385.44	13255.85 ±1651.33	p= 0.228
GSH-Px (U/gHb)	18.269 ± 2.527	19.475 ± 3.228	p= 0.162
GR (U/gHb)	9.158 ± 1.401	9.584 ± 1.076	p= 0.218
$MDA_{eryt.}$ (µmol/gHb)	0.289 ± 0.044	0.302 ± 0.044	p= 0.223
$MDA_{plas.}$ (µmol/L)	0.740 ± 0.190	0.834 ± 0.195	p= 0.091
E-vit. (mg/dl)	0.917 ± 0.130	0.951 ± 0.112	p= 0.445

Table 5. Comparison of measured parameters of male individuals in patient and control groups.

	Control (n=16) $\overline{X}$ ±S	Patient (n = 27) $\overline{X}$ ± S	p value
SOD (U/gHb)	1184.51 ± 224.82	1762.21 ± 276.97	p< 0.001
CAT (U/gHb)	11385.13 ± 1601.85	12382.52 ± 2385.45	p= 0.152
GSH-Px (U/gHb)	18.432 ± 3.681	18.269 ± 2.527	p= 0.669
GR (U/gHb)	8.281 ± 1.297	9.158 ± 1.402	p= 0.023
$MDA_{eryt.}$ (µmol/gHb)	0.203 ± 0.048	0.289 ± 0.044	p< 0.001
$MDA_{plas.}$ (µmol/L)	0.423 ± 0.162	0.740 ± 0.190	p< 0.001
E-vit. (mg/dl)	1.073 ± 0.145	0.917 ± 0.130	p= 0.001

2003; Abraham et al., 2005; Kharrazi et al., 2008). However no study had been found about oxidative stress and oxidant/anti-oxidant system evaluation of CCHF patients in the existing literature. However, Celik et al. recently reported elevated serum xanthine oxidase activity in patients with CCHF which might be associated with ROS generated by the xanthine/xanthine oxidase system during inflammatory responses and elevated lipid peroxidation might contribute to cell damage and hemorrhage (Celik et al., 2010).

Table 6. Comparison of measured parameters of female individuals in patient and control groups.

	Control (n= 25) $\overline{X}$ ± S	Patient (n = 20) $\overline{X}$ ± S	p value
SOD (U/gHb)	1209.13 ± 215.66	1848.32 ± 227.32	p < 0.001
CAT (U/gHb)	12469.48 ± 1996.32	13255.85 ± 1651.33	p= 0.244
GSH-Px (U/gHb)	19.735 ± 4.079	19.475 ± 3.228	p= 0.945
GR (U/gHb)	9.250 ± 1.354	9.584 ± 1.076	p= 0.236
$MDA_{eryt.}$ (µmol/gHb)	0.212 ± 0.050	0.302 ± 0.044	p< 0.001
$MDA_{plas.}$ (µmol/L)	0.478 ± 0.165	0.834 ± 0.195	p< 0.001
E-vit. (mg/dl)	1.105 ± 0.278	0.951 ± 0.112	p= 0.003

As progress of the viral hemorrhagic fever disease caused by CCHF virus shows similarity to progress of the sepsis, results obtained from our results was compared to results of the researches done with viral infection and sepsis patients.

In subjects infected with hepatitis C, hepatitis B and HIV virus, during dengue hemorrhagic fever (DHF) and recurrent aphthous stomatitis (RAS) diseases SOD enzyme activity was found high in erythrocytes (Kaya et al., 2006; Gil et al., 2004; Look et al., 1997; Czuczejko et al., 2003; Altınyazar et al., 2006). Victor et al. found that ROS increase according to inflammation and this causes oxidative stress (Victor et al., 2005). Cherian et al. reported that in child subjects with sepsis, there was no difference between control and patient group and difference between two groups was not statistically significant when erythrocyte SOD enzyme activity was compared. These results explained with an adaptation developed against oxidative stress (Cherian et al., 2007).

In our study, SOD enzyme activity in patient group was found to be higher compared to control group erythrocytes (control group, 1199,52 ± 216,81 U/gHb; patient group 1798,85 ± 257,98 U/gHb). Difference between two groups was statistically significant (p=0.000). SOD enzyme activity in erythrocytes reported by Cherian et al shows similarity with our results found in CCHF patients (Cherian et al., 2007).

SOD activity is high in tissues that have high usage of oxygen. It had been thought that intracellular superoxide amount is increased as a result of increased NADPH oxidase enzyme of stimulated phagocytic leucocytes. At the same time, increased SOD activity, protects the oxygen metabolizing cells against the harmful effects of free radicals such as lipid peroxidation and take place in the intracellular termination of phagocyted bacteria. It can be thought that based on the increase of SOD enzyme activity, hydrogen peroxide can increase as well. Hydrogen peroxide is not a toxic molecule. However hydrogen peroxide can be transformed to hydroxyl radicals through Fentom and Haber-Weiss reactions. Formed hydroxyl radicals are highly toxic compounds since they cause lipid peroxidation. Living organisms have antioxidants and antioxidant enzyme systems that remove hydrogen peroxide before it is transforms to hydroxyl radicals (Zabłocka and Janusz, 2008). Excess hydrogen peroxide is removed by enzymes such as CAT, GSH-Px and GR (Manfredini et al., 2008; Ayar- Kayalı and Tarhan, 2004; Parihar et al., 2008). In our study when CAT, GSH-Px and GR enzyme activity of the erythrocytes of CCCF patient group is evaluated; it is determined that CAT enzyme activity is slightly higher compared to control group (control group 12046,32 ± 1907,92 U/gHb; patient group, 12754,15 ± 2129,11 U/gHb). However difference between two groups was not statistically significant (p=0.100). It is determined that patient erythrocytes have lower GSH-Px enzyme activity compared to control group (control group 19,226 ± 3,934 U/gHb; patient group 18,782 ± 2,877 U/gHb). Difference between two groups was statistically significant (p=0.209). It is determined that GR activity in patient erythrocytes is higher than control group erythrocytes (control group 8.872 ± 1.399 U/gHb; patient group, 9.342 ± 1.275 U/gHb). However, activity difference between two groups was not statistically significant (p=0.011).

In a study done with RAS patients it is reported that CAT activity is increased (Altınyazar et al., 2006). Even a small increase in GSH-Px enzyme activity is determined in Hepatitis C and Hepatitis B patients, it is reported that this increase is not statistically significant (Czuczejko et al., 2003). In a study done with Hepatitis B patients, a statistically significant decrease of GSH-Px and CAT enzyme activity is reported (Kaya et al., 2006). Another study done with DHF patients revealed that GSH-Px activity is low in these patients (Altınyazar et al., 2006). On the other hand, in a study contributed of HIV subjects an increase of GSH-Px activity of erythrocytes had been determined but this was not statistically significant (Look et al., 1997). Ko et al. reported that in Hepatitis C patients, GSH-Px enzyme activity is low, however there is an increase in GR and SOD enzyme activity and MDA levels. They indicated that blood MDA level, may vary between people as a result of GSH-Px and GR enzyme activities and interferon + ribavirin treatment, and immune cytokines that take place in immune system which are affected from HCV infection may be the cause of this differences (Ko et al., 2005). Karabulut et al. have evaluated SOD and CAT enzyme activities in 4 different groups: Control group contributed by healthy individuals,

patients with acute hepatitis C disease, untreated chronic hepatitis C patients and patients treated with interferon for six months. It is reported that, SOD enzyme activity is 19% higher in hepatitis C positive patients, SOD enzyme activity is 49% lower in chronic hepatitis C positive patients, 22% lower in chronic hepatitis C positive patients treated with interferon compared to control group. In chronic hepatitis C positive patients, CAT activity is reported 13% higher compared to control group and it was statistically significant. No statistically difference is reported between acute hepatitis C positive patient group and control group for CAT enzyme activity. CAT enzyme activity in acute hepatitis C positive patients is found 15% lower compared with interferon treated patients (Karabulut et al., 2002). CAT activity of acute hepatitis C positive patients reported by Karabulut et al. shows similarity with CAT activity of CCHF patients who contributed to our study. MDA, SOD and, GR results reported by Ko et al in chronic hepatitis C positive patients show similarity with our results of CCHF patients, however GSH-Px results were not similar.

In our study, no significant change of CAT and GSH-Px enzyme activity in CCHF patients determined compared to control group. Increase of SOD but decrease of CAT and GSH -Px activities may indicate that high amount of hydrogen peroxide which may have accumulated in the cells. Increased hydrogen peroxide may be transform to hydroxyl radicals and increase oxidative stress. High serum and erythrocyte MDA levels in CCHF patients support this suggestion.

Superoxide radicals are transformed to hydrogen peroxide by SOD enzyme. Hydrogen peroxide which is a result of this reaction is removed by CAT enzyme. Hydrogen peroxide molecules which are not removed can be transformed to hydroxyl radicals by Fentom and Haber-Weiss reactions. Hydroxyl radicals are very toxic compounds, and therefore balance of SOD/CAT is important.

Andrades et al. reported that ratio of SOD/CAT enzymes is increased in major organs such as heart, lungs, and kidneys after 11 hours in patients with lethal sepsis; however in patients with non-lethal sepsis SOD/CAT ratio increased in lungs and heart after 24 h and in kidneys after 96 h and none of this increases is as high as in patients with lethal sepsis, oxidative stress is different between lethal and non lethal sepsis and this is determined with SOD/CAT ratio in a study in which they evaluate differences between oxidative stress parameters in lethal and non-lethal sepsis subjects (Andrades et al., 2005). This may be related to cell damage during lethal sepsis. In our study it is seen that SOD activity is increased but CAT enzyme activity is not increased in CCHF patients. Therefore, a high SOD/CAT value was acquired. Hereby, it can be thought that a part of hydrogen peroxide is transformed to hydroxyl radicals which cause cell damage. It is obvious that wide research of SOD/CAT ratio with larger patient groups (age, sex, before/after certain treatment protocols, cure/death after treatment etc.) will contribute to better understanding of the pathogenesis of the disease and prognosis.

In our study, it was determined that vitamin E level is lower in CCHF patients compared to control group (control group 1,092 ± 0,233 mg/dl, patient group 0,931 ± 0,122 mg/dl). Difference between two group was found to be statistically significant (p=0.000) . Jordao et al. reported that in AIDS subjects, plasma vitamin E level is lower compared to control group, on the other hand vitamin E metabolite level is higher (Jordão et al., 1998). Pacht et al. analyzed vitamin E levels in 121 HIV positive patients with no lung problem history for a year. They determined that in these patients, vitamin E levels were low. Vitamin E levels are measured at the start of the prognosis and after 12 months later and found out that there is a significant amount of decrease at the end of 12 months (Pacht et al., 1997). Plasma vitamin E values obtained from AIDS patients and vitamin E levels obtained from CCHF patients from our study show correlation. As this decrease of vitamin E level in CCHF patient group may be a result of lack of vitamin intake, they may also decrease because of viral infection as determined by Pacht et al. or increased elimination of vitamin E via urine as determined by Jordao et al. We accept that non evaluation of vitamin E levels in the urine of CCHF patients as a mistake.

Meliva et al. determined that MDA - last product of lipid peroxidation- levels are increased and vitamin E levels are decreased in the rats which are injected influenza virus on the following 5th and 7th days of injection. In rats which are given vitamin E before virus injection, it is determined that lipid peroxidation products decreased and vitamin E levels increased at the end of the same period (Mileva et al., 2002). It is known that especially vitamin E from anti-oxidants is protective against influenza virus infection. Therefore vitamin E administration to CCHF during treatment may be useful.

In our study, it is found that plasma and erythrocyte MDA levels are higher compared to control group (control group plasma MDA level 0.457 ± 0.164 µmol/L, patient group 0.784 ± 0.196 µmol/L; control group erythrocyte MDA level 0,210 ± 0,048 µmol/gHb, patient group 0,290 ± 0,048 µmol/gHb). Difference between two groups was statistically significant (p=0.000). It is reported that MDA level in chronic hepatitis C subjects erythrocyte, serum MDA level in HIV positive children, plasma MDA level in HIV/AIDS patients, serum MDA levels in hepatitis B positive patients, and colon, uterus and liver MDA levels of rats which sepsis is formed are higher compared to control group (Ko et al., 2005; Kılıç et al., 2005; Gil et al., 2003; Iseri et al., 2005). In this study, MDA levels determined in patients with viral infection and sepsis and MDA levels determined in erythrocytes and plasma in our study shows parallelism. So it can be told that oxidative stress is increased in CCHF patients. As a result, in order for oxidative stress to cause tissue and cell damage, oxidant

production should increase anti-oxidant capacity or anti-oxidant systems should fail. In this study it had been seen that SOD enzyme activity is increased in CCHF patients, however CAT, GSH-Px and GR enzyme activities are not changed in statistical significance. Vitamin E level determined is lower in patient group. In our study, the determination of high values of SOD activities and MDA values make us think that there is an increase in free oxygen radicals and lipid peroxidation in CCHF patients.

With the information gathered from our study, it can be foreseen that oxidant/anti-oxidant level may play an important role in pathogenesis of CCHF disease and because the primary treatment of CCHF is supporting therapy in order to improve prognosis nutrition with anti-oxidant included diet or usage of anti-oxidant included foods may be useful. Since vitamin E levels are found low in these patients, addition of vitamin E to the treatment may be useful as well.

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