Serum Ceruloplasmin Oxidase Activity and Malondialdehyde Level in Experimental Abdominal Compartment Syndrome-Induced Rats

Deneysel Abdominal Kompartman Sendromu Oluşturulan Sıçanlarda Serum Malondialdehid Düzeyi ile Seruloplazmin Oksidaz Aktivitesi

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ABSTRACT Objective: The aim of this study was to investigate serum ceruloplasmin (Cp) oxidase activity and malondialdehyde (MDA) levels in a rat model of abdominal compartment syndrome (ACS). **Material and Methods:** There were four groups consisting of eight rats per group. Group 1 (Control group): After anesthesia, 3 ml of blood were taken from the heart of these rats. Group 2 (Sham group): Under anesthesia, an injector needle was inserted intraperitoneally and left there. After 3 h, 3 ml of blood was taken from the heart of these rats. Group 3 (First study group): Under anesthesia, an injector needle (number 16) was inserted intraperitoneally and a standard insufflator (Karl Storz, Germany) was connected. At the end of this procedure, the abdomen was decompressed and 3 ml of blood was collected as descried above. Group 4 (Second study group): The abdomen was compressed as Group 3. However, the intraabdominal hypertension was established by keeping the pressure constant at 25 mmHg for 3 h in this group. At the end of this procedure, the abdomen was decompressed and 3 ml of blood was collected as descried above. **Results:** Cp oxidase activities and MDA levels significantly increased in first and second study groups when compared to the control group. Cp oxidase activities and MDA levels were significantly higher in second study group than those of the first study group. MDA levels were significantly higher in first study group than those of the sham group. **Conclusion:** We found increased serum Cp oxidase activity and MDA level in experimental abdominal compartment syndrome-induced rats. These findings show that oxidative stress increase ACS in rats.

Key Words: Ceruloplasmin; compartment syndromes

ÖZET Amaç: Bu çalışmanın amacı abdominal kompartman sendromu (AKS) oluşturulan sıçanlarda serum seruloplazmin (Cp) oksidaz aktivitesi ve malondialdehit (MDA) seviyelerini araştırmaktır. **Gereç ve Yöntemler:** Çalışmada her biri sekizer denekten oluşan dört grup oluşturuldu. Grup 1 (Kontrol grubu): Deneklerde anesteziyi takiben intrakardiyak 3 cc kadar kan alındı. Grup 2 (Sham grubu): Anestezi altında peritoneal boşluğa 16 numara enjektör iğnesi ile girildi ve iğne yerinde bırakıldı. Üç saat beklendikten sonra deneklerden aynı yöntemle kan alındı. Grup 3 (Birinci çalışma grubu): Anestezi altında peritoneal boşluğa 16 numara enjektör iğnesi ile girildi ve bunun ucuna standart bir insuflatör (Karl Storz, Germany) cihazı bağlandı. İnsuflatör yardımıyla batın CO2 gazıyla şişirildi. İnsuflatör 20 mmHg basıncında sabit tutularak 3 saat süreyle AKS oluşturuldu. İşlem sonunda batın desufle edildi. Kan örnekleri aynı yöntemle alındı. Grup 4 (İkinci çalışma grubu): Üçüncü gruptaki gibi batın insufle edildi. Ancak bu grupta insuflatör 25 mmHg basıncında sabit tutularak 3 saat süreyle AKS oluşturuldu. İşlem sonunda batın desufle edildi. Kan örnekleri aynı yöntemle alındı. **Bulgular:** Cp oksidaz aktivitesi ve MDA seviyeleri, kontrol grubu ile karşılaştırıldığında birinci ve ikinci çalışma grubunda önemli derecede artmıştı. İkinci çalışma grubundaki Cp oksidaz aktivitesi ve MDA seviyeleri, birinci çalışma grubundan daha yüksekti. Birinci çalışma grubundaki MDA seviyeleri, sham grubundan daha yüksekti. **Sonuç:** AKS'lu sıçanlarda MDA düzeyleri ile Cp oksidaz aktivitesinin arttığını bulduk. Bu bulgular AKS oluşturulan sıçanlarda oksidatif stresın arttığını göstermektedır.

Anahtar Kelimeler: Seruloplasmin; kompartman sendromları

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Intra-abdominal hypertension (IAH) and the abdominal compartment syndrome (ACS) are well-recognized pathological entities that frequently occur in critically ill patients and indicate a worsening of the prognosis, although there is still a lack of awareness among physicians with regard to this problem.[1,2] Intraabdominal pressure (IAH) may be acutely increased by a variety of causes after major trauma or abdominal surgery. The detrimental effects on cardiac, pulmonary, hepatic and renal systems with raised IAP and ACS are well known and easy to detect clinically. The ensuing organ dysfunction often resolves following surgical decompression of the abdomen; however, this may then lead to complications which can cause serious additional morbidity.[3]

Reactive oxygen species (ROS), generated in the organism as byproducts of normal cellular metabolism have been implicated in the pathogenesis of a large number of diseases such as diabetes mellitus, cancer, rheumatoid arthritis, infectious diseases, atherosclerosis and aging.[4,5] Although ROS have several physiological functions in signal transduction, gene transcription and regulation, they are able to cause oxidation of biomolecules, thereby contributing to their structural and functional modifications. This leads to cell dysfunction and cell death, and, at the organic level, to ageing and age-related diseases. Many enzymatic and nonenzymatic antioxidants have been developed by aerobic organisms to counteract the effects of ROS on biomolecules.[6] Ceruloplasmin (Cp) is an alpha-2-glycoprotein that contains more than 95% of the copper in the plasma and plays an important role in iron homeostasis. Other roles include its participation in the antioxidant defense or in oxidative damage mechanisms and its involvement in a number of processes related to the metabolism of copper, and biogenic amines, and nitric oxide.[7-10] Under physiologic conditions, Cp oxidase is also important in the control of membrane lipid oxidation, probably by direct oxidation of cations, thus preventing their catalysis of lipid peroxidation.[4,5]

The process of lipid peroxidation is oxidative conversion of polyunsaturated fatty acids to products known as malondialdehyde (MDA), which is usually measured as thiobarbituric acid reactive substances (TBARS), or to lipid peroxides, which is the most studied, biologically relevant, free radical reaction. Lipid peroxidation of cellular structures, a consequence of free radical activity, is thought to play an important role in aging, atherosclerosis and late complications of diabetes.[5]

To our knowledge, there is no study that investigates simultaneously Cp oxidase activity and malondialdehyde (MDA) levels, an indicator of lipid peroxidation, in a rat model of ACS. Therefore, in the present study, we aimed to investigate Cp oxidase activity and malondialdehyde (MDA) levels in this experimental model.

MATERIAL AND METHODS

In the study, 32 male Sprague-dewlay rats (200-235 g), which were fed with standard diet and kept at the same conditions, were used. All animals received humane care in compliance with the guidelines of Ataturk University Research Council's criteria. The rats fasted for 12 h before the experiment.

There were four groups consisting of eight rats per group. Ketamine HCL (85 mg/kg) and xylazine (6 mg/kg, intramuscular) were used to anesthetise rats.

Group 1 (Control group): After anesthesia, 3 ml of blood was taken from the heart of these rats.

Group 2 (Sham group): Under anesthesia, an injector needle was inserted intraperitoneally and left there. After 3 h, 3 ml of blood was taken from the heart of these rats.

Group 3 (First study group): Under anesthesia, an injector needle (number 16) was inserted intraperitoneally and a standart insufflator (Karl Storz, Germany) was connected. The abdomen was inflated with carbon dioxide (CO_2). Intraabdominal hypertension was established by keeping the pressure constant at 20 mmHg for 3 h in this group. At the end of this procedure, the abdomen was decompressed and 3 ml of blood was collected as descried above.

Group 4 (Second study group): The abdomen was compressed as in Group 3. However, the intra-abdominal hypertension was established by kee-

ping the pressure constant at 25 mmHg for 3 h in this group. At the end of this procedure, the abdomen was decompressed and 3 ml of blood was collected as descried above.

BIOCHEMICAL ANALYSIS

The blood samples obtained were transferred to vacutainers and left for 30 min at room temperature. They were centrifuged at 3000xg for 10 min. Serum was collected as two aliquots and kept at -80°C until biochemical analyses.

Serum MDA was determined by the thiobarbituric acid method.[11] Serum aliquots (0.2 ml) were mixed thoroughly with 0.8 ml of phosphatebuffered saline (pH 7.4) and 0.025 ml of butylated hydroxytoluene solution. After addition of 0.5 ml of 30% trichloroacetic acid, the samples were placed on ice for 2 hr and then centrifuged at 2000 x g at 25°C for 15 min. One ml of supernatant was mixed with 0.075 ml of 0.1 mol/L EDTA and 0.25 ml of 1% thiobarbituric acid in 0.05 N sodium hydroxide. The samples were placed in boiling water for 15 min, cooled to room temperature, and the absorbance was determined at 532 nm. Total thiobarbituric acidreactive substances (TBARS) were expressed as MDA. MDA levels were expressed as μmol/L.

Serum Cp oxidase activity was measured according to the method of Schosinski et al.[12] The method is based on the ability of ceruloplasmin to oxidize substrate such as o-dianizidine (3,3,-dimethoxybenzidine) yielding a yellow product. Briefly, 0.75 ml of 0.1 M acetate buffer, pH 5, in two tubes was mixed with 0.05 ml of serum sample and kept for 5 min at 30°C. To both tubes, 0.2 ml of 0.25% o-dianizidine dihydrohloride was added and one mixture was incubated at 30°C for 5 min, and the other for 15 min. The reaction was stopped by adding 2 ml of 9 M sulfuric acid. The optical density was determined at 540 nm using a spectrophotometer (CECIL CE 3041, Cambridge, UK). Cp oxidase activity was expressed as U/L.

STATISTICAL ANALYSIS

The findings were expressed as the mean ± SD. Normality distribution was assessed using Shapiro-Wilk test. All variables were normally distributed. Statistical analysis was undertaken using one way ANOVA with Fisher's LSD Multiple-Comparison test. A p value < 0.05 was accepted as statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 11.0, Chicago, IL, USA).

RESULTS

All parameters are shown in Table 1. As seen from the Table, serum Cp oxidase activities and MDA levels significantly increased in the first and second study groups when compared to the control group. Serum Cp oxidase activities and MDA levels were significantly higher in second study group than those of the first study group. Serum MDA levels were significantly higher in first study group than those of the sham group.

DISCUSSION

There are many clinical situations that can lead to increased IAH, which, in turn, can cause fatal multiple organ failure called ACS. Causes of ACS include tense ascites, abdominal hemorrhage, intestinal obstruction, large abdominal tumors and peritoneal dialysis. In addition, permanent gas insufflation, which is used commonly during laparoscopic surgery to provide intraabdominal working space, elevates IAH immensely. Changes in splanchnic blood flow and hepatic afferent circulation, due to the elevation of intraabdominal pressure accompanying CO_2 pneumoperitoneum, have also been described.[3,13-15]

TABLE 1: Serum ceruloplasmin oxidase activity and lipid peroxidation in experimental abdominal compartment syndrome in rats (Mean± SD).

	Cp oxidase (IU/L)	MDA (μmol/L)
Group 1 (Control group)	182.6 ± 25.7	1.9 ± 0.8
Group 2 (Sham group)	195.7 ± 8.6	3.2 ± 1.1[a]
Group 3 (First study group)	204.3 ± 3.9[a]	4.7 ± 1.4[b,c]
Group 4 (Second study group)	221.1 ± 4.4[b,c,e]	6.3 ± 1.5[b,d,f]

a: p<0.05, b: p<0.001, vs. control group,
d: p<0.01, e:p<0.001 vs. sham group,
f: p<0.05, g: p<0.01, vs. first study group.
There were 7 animals in each group

Oxidants such as superoxide radical ($O^{\cdot-}_2$) hydroxyl radical ($OH^{\cdot-}$) are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms. Oxidative stress is an imbalance between the production of free radicals that contain unpaired electrons and antioxidant defences buffering the oxidative damages. Oxidative effects of free radicals are controlled by exogenous antioxidants such as vitamins E and C, and also by endogenous antioxidants. Under some conditions, an increase in oxidants and a decrease in antioxidants cannot be prevented, and oxidative/antioxidative balance shifts towards the oxidative stress.[16-18]

Cp is a blue copper oxidase that is synthesized by hepatocytes and secreted as a holoprotein with six atoms of copper incorporated during the biosynthesis.[19] Nevertheless, as indicated by considerable experimental evidence, it is particularly prone to transfer its copper atoms to tissues delivering copper to intracellular copper proteins. However, recent studies on aceruloplasminemic patients indicate that this protein has no essential role in copper transport, whereas it plays a primary role in iron homeostasis, possibly through its ferroxidase activity.[10,18] Antioxidant activity of Cp can be ascribed mainly to its ferroxidase activity, which inhibits ferrous ion-stimulated lipid peroxidation and formation of $OH^{\cdot-}$ in the Fenton reaction. Cp is not only a ferroxidase but also a scavenger of ROS. In aceruloplasminemia, many reports showed a marked increase in lipid peroxidation in cerebral spinal fluid and brain tissues of patients.[19,20]

In this study, we found that serum Cp oxidase activity was significantly higher in first and second study groups than that of the control group. Of the extracellular antioxidants, Cp oxidase oxidizes Fe^{2+} to Fe^{3+} and facilitates binding of ferric iron, Fe^{3+}, to transfferin (Trf). Trf inhibits iron-ion dependent $OH^{\cdot-}$ formation from hydrogen peroxide (H_2O_2).[4,5] Increased Cp oxidase activity may be a protective response to an increase in circulating unbound Fe^{2+} due to increased oxidative stress in response to ACS-induced rats in our study, which act as a catalyst for further free radical-induced lipid peroxidation.

It has been reported that ROS played a role in the pathogenesis of a number of diseases. ROS are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, protein, free amino acids, lipids and lipoproteins, carbohydrates, and connective tissue macromolecules. These species may impair cell activities such as membrane function, metabolism, and gene expression. Propagation of damage results in a repeated chain reaction. When the balance between ROS production and the antioxidative defense mechanisms is impaired, ROS levels may increase. When ROS are not removed by natural scavengers, damage occurs through peroxidation of structurally important polyunsaturated fatty acid within the phospholipid structure of the membranes. Lipid peroxidation decreases both the fluidity and the barrier function of membranes, resulting in disturbances in structural organization, enzymic inhibition, and possible cell death. In addition, lipid peroxides may inhibit protein synthesis, block macrophage function, and alter chemotactic activity.[4,5,21,22]

When ACS develops, it is mandatory to decompress the abdomen surgically as soon as possible. Although this causes reperfusion of abdominal organs and it also produces arterial hypotension. It is well known that reperfusion of the ischemic tissue may promote the generation of ROS, which are known to have deleterious effects on various cellular functions.[1,24] The organ dysfunction that accompanies this condition is generally associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, parenchimal cell dysfunction and necrosis.[1,25] Ischemia/reperfusion elicits an acute inflammatory response characterized by activation of neutrophils. Activated neutrophils are known to induce tissue injury through the production and release of ROS. Lipid peroxidation, as a free radical generating system, has been suggested to be closely related to ischemia-reperfusion-induced tissue damage, and MDA an important indicator of lipid peroxidation.[1,23-26] In the study, we found that serum MDA levels were significantly higher in first and second study groups when com-

pared to the control group in experimental ACS. Our results for MDA values, which is an important indicator of oxidant stress, are in agreement with that obtained by Sener et al.[24]

In conclusion, this is the first study that investigates simultaneously Cp oxidase activity and MDA levels, an indicator of lipid peroxidation, in experimental ACS-induced rats. We found the increased Cp oxidase activity and MDA level were significantly higher in first and second study groups when compared to the control group. These results suggest that ROS play a role in ACS in rats. Therapy with antioxidants may lead to the increase in the antioxidant defense system and thus improvement in clinical symptoms in ACS. Further studies including determination of 4-hydroxy-2-nonenal (HNE), a more specific peroxidation product of essential fatty acids, particularly arachidonic acid, in addition to MDA and Cp oxidase assay are needed to provide definitive information about the relationships between lipid peroxidation and antioxidant system in ACS.

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