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Original Article

The Effect of Extracorporeal Circulation on Oxidative Stress and Viscosity

Osman Fuat Sönmez,[1] Çiğdem Özer,[2] Deniz Erbaş,[3] Mehmet Altan,[1] Gürsel Levent Oktar,[4] Halim Soncul[4]

[1]Department of Physiology, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, İstanbul, Türkiye
[2]Department of Physiology, Gazi University Faculty of Medicine, Ankara, Türkiye
[3]Department of Physiology, University of Kyrenia Faculty of Medicine, North Cyprus
[4]Department of Cardiovascular Surgery, Gazi University Faculty of Medicine, Ankara, Türkiye

Abstract

Objective: One of the main determinants of morbidity and mortality after cardiopulmonary bypass (CPB) and its associated extracorporeal circulation (EC) is the oxidant stress that occurs during and after the procedures. There are unknowns about mechanisms that need to be revealed. This study aimed to examine the effecs of EC on oxidant, antioxidant parameters, and viscosity.

Methods: The study was carried out in 22 patients between the ages of 42 and 70 undergoing Coronary artery bypass grafting. Blood was drawn at seven different stages of the operation, including A: Pre-operative intensive care stage, B: Anesthesia, C: Connecting to the pump, D: After aortic cross-clamp, E: Release of the aortic cross-clamping, F: Disconnection from the pump, and G: Post-operative intensive care. Malonyldialdehyde (MDA) and Nitrite + Nitrate (NOx) levels were measured in terms of oxidant stress, and total sulfhydryl group (RSH) levels as an antioxidant indicator. Furthermore, plasma viscosity at each stage was measured.

Results: At the C stage, NOx and MDA levels increased, and RSH levels began to decrease. The highest MDA, NOx, and lowest RSH levels were detected in the F stage. Viscosity was also decreased after connection to the pump and increased after pump disconnection.

Conclusion: MDA and RSH levels were compatible with oxidant stress and antioxidant capacity reduction, respectively, caused by surgical trauma and non-physiological surfaces of EC. One of the reasons for the increase in NOx levels is the mechanical stimulation of the endothelium as a result of surgical procedures. Another reason may be that the erythrocytes circulating on the artificial surfaces of EC are exposed to mechanical stress and their eNOS expression. The decreased viscosity develops due to hemodilution and plasma protein denaturation.

Keywords: Extracorporeal circulation; malonyldialdehyde; RSH; total nitric oxide; viscosity.

Ekstrakorporal Dolaşımın Oksidatif Stres ve Vizkosite Üzerine Etkisi

Özet

Amaç: Kardiyopulmoner bypass (KPB) ve buna bağlı ekstrakorporeal dolaşım (ED) nedeniyle gelişen oksidatif stres, morbidite ve mortalitenin ana belirleyicilerinden biridir. ED'nin oksidatif stres ve viskoziteye etkisini incelemeyi amaçladık.

Yöntem: Çalışmamıza 42-70 yaşları arasında KPB grefti uygulanan 22 hasta dahil edildi. Ameliyatın yedi farklı aşamasında kan alındı: A: Ameliyat öncesi yoğun bakım aşaması, B: Anestezi, C: Pompaya bağlanma, D: Aort kros klemp sonrası, E: Aort kros klempinin kaldırılması, F: Pompadan ayrılma, G: Ameliyat sonrası yoğun bakım. Oksidan stres açısından malondialdehit (MDA) ve nitrit + nitrat (NOx) düzeyleri ve antioksidan gösterge olarak toplam sülfidril bileşikleri (RSH) düzeyleri ölçüldü. Her aşamada plazma viskozitesi ölçüldü.

Bulgular: C evresinde MDA ve NOx seviyeleri önemli ölçüde artarken, RSH seviyeleri önemli ölçüde azaldı. En yüksek MDA, NOx ve en düşük RSH seviyeleri F evresinde tespit edildi. Viskozite de pompaya bağlandıktan sonra azaldı ve pompa bağlantısı kesildikten sonra arttı.

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Address for Correspondence: Osman Fuat Sönmez

Department of Physiology, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, İstanbul, Türkiye

E-mail: osmanfuat.sonmez@iuc.edu.tr, ofuats@gmail.com

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Sonuç: MDA ve RSH seviyeleri, cerrahi travma ve endotelyal hücrelerin fizyolojik olmayan yüzeylerinden kaynaklanan oksidan stres ve antioksidan kapasite azalmasıyla uyumluydu. NOx seviyelerindeki artışın nedenlerinden biri, cerrahi işlemler sonucu endotelin mekanik olarak uyarılmasıdır. Bir diğer neden ise, endotelyal hücrelerin yapay yüzeylerinde dolaşan eritrositlerin mekanik strese maruz kalması ve eNOS ekspresyonunun artması olabilir. Viskozite azalması, hemodilüsyon ve plazma protein denatürasyonuna bağlı olarak gelişir.

Anahtar sözcükler: Ekstrakorporeal dolaşım; malondialdehit; RSH; toplam nitrik oksit; viskozite.

Introduction

Today, with modern diagnosis methods, the frequency of diagnosis of coronary artery disease and related coronary artery bypass operations is increasing. Furthermore, an increase in average life expectancy has caused an increase in cardiovascular events and, therefore, cardiac surgical interventions. During these interventions, extracorporeal circulation (EC) is frequently provided to facilitate surgery and to ensure adequate perfusion for the other organs. However, despite advanced technology and applications, various complications may develop in various organs, including hyperglycemia, dyslipidemia, blood damage, and hemolysis.[1–3]

Systemic inflammatory response syndrome (SIRS) is triggered in the patient during cardiac surgery performed by cardiopulmonary bypass (CPB). The induction of CPB-related inflammation occurs by the activation of many pathways, such as the complement system, coagulation-fibrinolysis cascade, cytokines, endothelial and cellular immune systems. In the literature, possible causes of the SIRS, such as the contact of blood with the inner surface of the pump system that provides the "Extracorporeal circulation" during CPB, the presence of ischemia-reperfusion (I/R), anesthesia, endotoxemia, surgical stress, and hypothermia has been reported.[4,5]

Theories of inflammation-induced cell damage are frequently based on neutrophil and leukocyte activation. Neutrophil activation causes the release of reactive oxygen species (ROS), intracellular proteases, and arachidonic acid metabolites. Increased levels of ROS species, such as hydrogen peroxide, hydroxyl radicals, and superoxide anions, are released from activated neutrophils. Lipid peroxidation products such as MDA reflect the severity of ROS that is effective in tissue damage.

These released products trigger inflammatory responses and may be the cause of post-operative complications such as myocardial dysfunction, respiratory failure, renal and neurological disorders, liver dysfunction, bleeding diathesis, and even multiple organ failure.[6–8]

Due to its important role in patient morbidity and mortality, various methods are used to reduce the inflammatory process that occurs in response to the negative effects of CPB. In addition to pharmacological agents such as glucocorticoids, protease inhibitors, phosphodiesterase inhibitors, antioxidants, heparin, sodium nitroprusside, complement inhibitors, and monoclonal antibodies, heparin coating of CPB circuits, changing pump flow strategies, and application of ultrafiltration during CPB have been used in the clinic.[8–10]

It is noteworthy that EC's relations with lipid peroxidation and the antioxidant system are emphasized. However, in these studies, oxidant stress was examined intensively before and after CPB and I/R, and the findings were not sufficient to explain the mechanism. It has also been observed that studies on changes in viscosity during EC are insufficient. The effect of non-physiological environmental factors on the blood during CPB surgery is important.

In our study, it was aimed to get blood samples at every stage of CPB and administer EC, to examine oxidant, antioxidant parameters, and viscosity changes.

Materials and Methods

Study Population

The study was performed on 22 patients (10 females and 12 males) aged between 42 and 70 years who underwent coronary bypass surgery. Before the experiment, Gazi University University Faculty of Medicine Local Ethics Committee permission was obtained (Date: 26.05.2008, Decision no: 199). The patients were informed about the procedures to be carried out in line with the ethical rules, and those who accepted to participate by reading the "Volunteer Consent Form" were included in the study. The mean age of the patients participating in the study was 62.5±, and all cases were diagnosed with coronary artery disease with 2 or 3 vessels. Standard anesthesia protocol was carried out. After anesthesia, patients were connected to the heart-lung machine. Anesthesia induction was standardized, and all patients were provided with remifentanil 0.5 μg/kg. Continued with 2 mg/kg propofol, 0.1 mg/kg pancuronium was administered as a muscle relaxant. In patients who were intubated, ventilation was provided using a volume-controlled ventilator (Tbird AVS-3, Model 15586-C; Bird Products Corp, Palm Springs, CA). For the maintenance of anesthesia, 0.25–0.50 μg/kg/h remifentanil, 2 mg/kg/h propofol, and isoflurane (0.4–1%) by inhalation were administered. Ascending aorta cannulation and two-stage right atrium venous cannulation were performed after standard sternotomy. Anticoagulation was provided with heparin (3.0 mg/kg). The activated clotting time was monitored as 400±10 s. Body temperature was lowered between 28°C and 32°C to achieve mild hypothermia. Cold blood cardioplegia was applied to all patients. Cardioplegia solution was prepared using 40 mEq of KCl, 20 mEq of $NaHCO_3$, and 0.5 mL of Ca Gluconate (0.9%) in 500 mL isotonic saline. Following aortic cross-clamping, blood cardioplegia solution (15 mL/kg) was infused and repeated at 5 mL/kg dose at every 20 min intervals. A membrane oxygenator (Jostra VKMO 4200; Quadrox-VHK 4200) was used to achieve extracorporeal oxygenation. A roller pump was used for EC, and continuous current was applied (Stöckert, Munich, Germany). The total flow upper limit was set to be m^2 (body surface area) × 2.4 L/m^2/min and to ensure adequate and continuous organ perfusion.

To observe and compare the effects of the applications performed in CPB gradually, blood samples were taken from the patients at seven different stages.

- Stage I (A): In intensive care (before the operation),
- Stage II (B): Under anesthesia,
- Stage III (C): Pump in (after circulation connected to the pump),
- Stage IV (D): After the aortic cross clamp placed,
- Stage V (E): Aortic cross clamp removed,
- Stage VI (F): Pump out (when patients' circulation leaves the EC system),
- Stage VII (G): In intensive care (after the operation).

The mean duration at the pump was 83 min (minimum 46 min and maximum 129 min), and the mean aortic cross-clamp time was 36 min (minimum 21 min and maximum 54 min). During the procedures listed above, 8 mL of blood samples were taken from the patients participating in the study. Blood was taken from the venous system in intensive care (before the operation). It was taken from the arterial system under Anesthesia, pumped out, and in an intense care (post-operative) period. In other stages, it was taken from the arterial line of the pump.

The samples taken were centrifuged at 3000 rpm for 10 min, and their plasma was separated. MDA, nitrite + nitrate (NOx) levels, and antioxidant RSH levels, which are indicators of oxidant stress in plasma, were studied by the spectrophotometric method. Blood viscosity was measured with the Vilastic Bioprofiler (Vilastic Scientific, Inc, Austin, U.S.).

Biochemical Determinations

Determination of plasma lipid peroxide level

Lipid peroxide levels in the perfusate were estimated using the method described by Kurtel et al.[11] based on the measurement of thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation. The perfusate was incubated at room temperature for 15 min on the day of the experiment. Following incubation, 0.5 mL of the supernatant was mixed with 1 mL of a solution containing 15% (w/v) trichloroacetic acid, 0.25 N HCl, and 0.375% (w/v) thiobarbituric acid. Protein precipitates were removed by centrifugation, and the resulting supernatants were transferred to glass test tubes containing 0.02% (w/v) butylated hydroxytoluene. The samples were then heated in a boiling water bath at 100°C for 15 min, cooled, and centrifuged again to eliminate any remaining precipitates. The absorbance of each sample was measured at 532 nm. Lipid peroxide levels were expressed as MDA equivalents, using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Determination of plasma RSH level

RSH levels were determined using the method described by Kurtel et al.[11] A 0.5 mL aliquot of each sample was mixed with 1 mL of a solution containing 100 mM Tris-HCl (pH 8.2), 1% sodium dodecyl sulfate, and 2 mM ethylenediaminetetraacetic acid. The mixture was incubated at 25°C for 5 min and then centrifuged to remove any precipitate. Subsequently, 0.3 mM 5,5-dithio-bis-2-nitrobenzoic acid was added to each reaction mixture and incubated at 37°C for 15 min. The absorbance of each sample was measured at 412 nm, and RSH levels were calculated using a molar extinction coefficient of 13,000 $M^{-1}\cdot cm^{-1}$.

Determination of plasma NOx levels

NOx levels were obtained from an Enzyme-Linked Immunosorbent Assay (ELISA) reader by vanadium III chloride (VCl_3)/Griess assay. After centrifugation, the plasma was separated. Plasma samples were deproteinized using 0.3 M NaOH and 5% (w/v) $ZnSO_4$, followed by centrifugation at 14,000 rpm for 5 min. The resulting supernatants were collected and used for the assays.[12]

The nitrate standard solution was serially diluted. After loading 100 µl of each sample into the wells, 100 µL of VCl_3 was added to each well, immediately followed by the addition of Griess reagents: 50 µL of sulphanilamide and 50 µL of N-(1-naphthyl)ethylenediamine dihydrochloride. Following incubation for 30–45 min, absorbance was measured at 540 nm using an ELISA reader.

Viscosity Measurement

It was measured using oscillatory flow in a rigid, cylindrical tube with the instrument of Vilastic Bioprofiler (Vilastic Scientific, Inc., Austin, U.S.). Before the measurement, the measuring tube of the device was filled with deionized water and set to 37°C. When the temperature equilibrium was achieved, the viscosity of the deionized water was measured, and the blood sample to be loaded was subtracted from its viscosity. Thus, the effect of the carrier fluid on sample measurement is eliminated. All measurements were made under constant temperature. To achieve this, the device was provided to operate at a constant temperature for at least 40 min before measurement. As a result, a comparison was made by measuring viscosity, elasticity, and shear rate.

Statistical Analysis

All data were presented as mean values ± standard deviation. Differences among the three groups were analyzed by one-way analysis of variance. Mann–Whitney U test was used for pairwise comparisons among groups. The accepted level of significance was set at $p < 0.05$. Data were analyzed with the statistical package Statistical Package for the Social Sciences (SPSS) for Windows (version 13.0, SPSS Inc., Chicago, IL, USA).

Results

Plasma TBARS Levels

Plasma MDA levels started to increase from the moment the circulation entered the pump. Pump in (C) and aortic clamping (D) stages did not differ significantly in terms of MDA levels among themselves. The most significant increases were detected in the clamp removal and pump output (E and F) stages. Although MDA levels showed a significant decrease in the measurements made in the post-operative intensive care (G) stage, it was higher than the values in the pre-operative intensive care (A) and anesthesia (B) periods (Fig. 1).

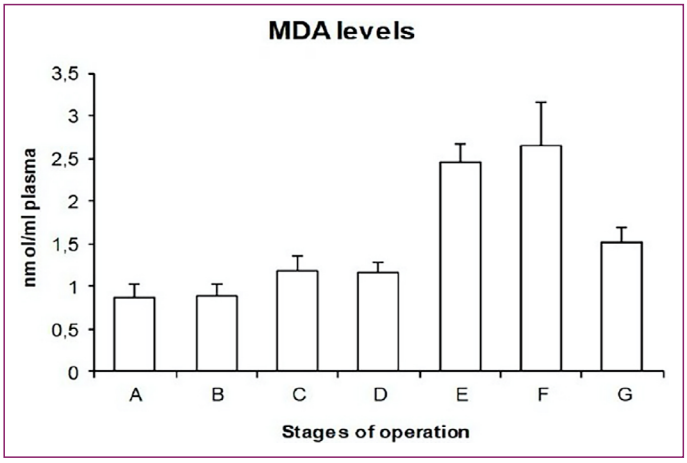

Figure 1. Plasma malonyldialdehyde levels. (a) Pre-operative intensive care stage, (b) During anesthesia, (c) After pump-in stage, (d) After aortic cross-clamping stage, (e) After release of the aortic cross-clamping stage, (f) After pump-out stage, (g) Post-operative intensive care stage.

All data were presented as mean values±standard deviation. Differences among the seven groups were analyzed by one-way analysis of variance. The Mann-Whitney U Test was used for pairwise comparisons among groups. p<0.01: E-F; p<0.0001: A-C, A-D, A-E, A-F, A-G, B-C, B-D, B-E, B-F, B-G, C-E, C-F, C-G, D-E, D-F, D-G, E-G, F-G.

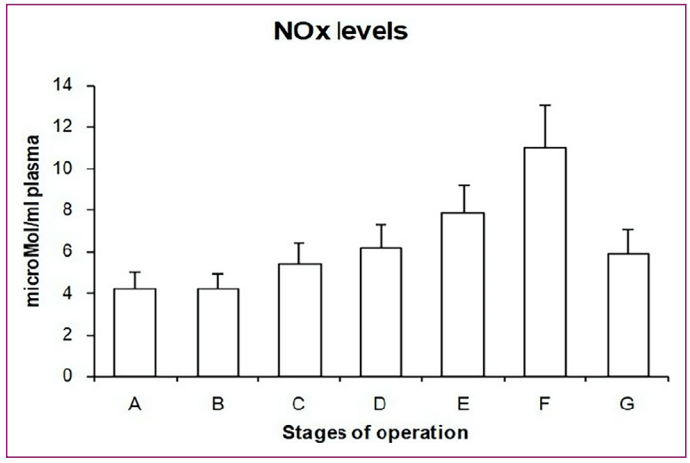

Figure 3. Plasma nitrite + nitrate levels. (a) Pre-operative intensive care stage, (b) during anesthesia, (c) after pump-in stage, (d) after aortic cross-clamping stage, (e) after release of the aortic cross-clamping stage, (f) after pump-out stage, (g) post-operative intensive care stage.

All data were presented as mean values±standard deviation. Differences among the seven groups were analyzed by one-way analysis of variance. The Mann–Whitney U Test was used for pairwise comparisons among groups. p<0.05: C-D; p<0.01: B-C; p<0.0001: A-C, A-D, A-E, A-F, A-G, B-D, B-E, B-F, B-G, C-E, C-F, D-E, D-F, E-F, E-G, F-G.

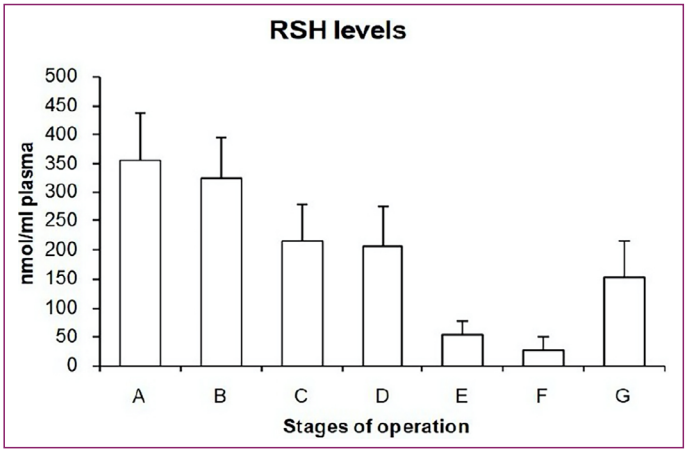

Figure 2. Plasma RSH levels. (a) Pre-operative intensive care stage, (b) During anesthesia, (c) After pump-in stage, (d) After aortic cross-clamping stage, (e) After release of the aortic cross-clamping stage, (f) After pump-out stage, (g) Post-operative intensive care stage.

All data were presented as mean values±standard deviation. Differences among the seven groups were analyzed by one-way analysis of variance. Mann–Whitney U Test was used for pairwise comparisons among groups. p<0.01; E-F, C-G; p<0.0001: A-C, A-D, A-E, A-F, A-G, B-C, B-D, B-E, B-F, B-G, C-E, C-F, D-E, D-F, D-G, E-G, F-G.

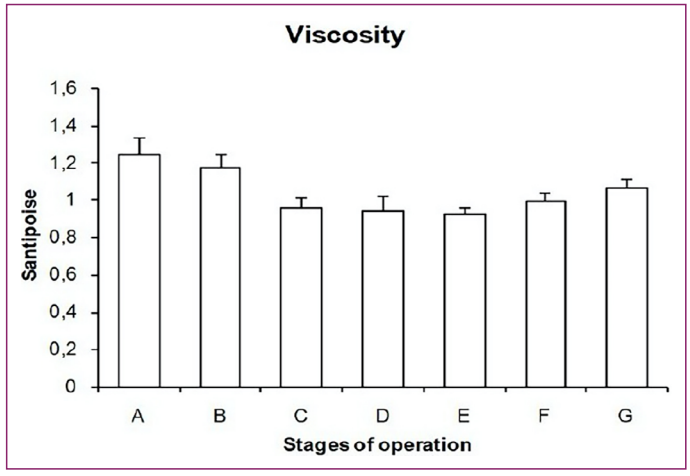

Figure 4. Plasma viscosity levels. (a) Pre-operative intensive care stage, (b) during anesthesia, (c) after pump-in stage, (d) after aortic cross-clamping stage, (e) after release of the aortic cross-clamping stage, (f) after pump-out stage, (g) Post-operative intensive care stage.

All data were presented as mean values±standard deviation. Differences among the seven groups were analyzed by one-way analysis of variance. The Mann–Whitney U Test was used for pairwise comparisons among groups. p<0.05: C-E; p<0.01: A-B, C-F, D-F; p<0.001: A-C, A-D, A-E, A-F, A-G, B-C, B-D, B-E, B-F, B-G, C-G, D-G, E-F, E-G, F-G.

Plasma RSH Levels

Plasma RSH levels decreased significantly after connection of the circulation to the pump. The lowest values were detected in the period when the aortic clamp was removed (E) and pump out (F) periods. In the post-operative intensive care (G) period, it was found to be significantly increased compared to the pump-out period (Fig. 2).

Plasma NOx Levels

Findings paralleled with the change in MDA levels. Plasma NOx levels began to rise significantly after the connection of the circulation to the pump. The highest levels were detected during the pump-out (F) period. A significant decrease was noted in the post-operative intensive care (G) stage (Fig. 3).

Plasma Viscosity Values

Viscosity decreased with the application of anesthesia. Decrement was more significant after the blood was connected to the pump. Although increased in the pump-out period, it was measured below the values of the first stages (stages A and B) (Fig. 4).

Discussion

CPB is one of the basic procedures of open-heart surgery. Despite the development of endovascular interventional techniques, CPB is still used in the treatment of many cardiovascular diseases that require surgery because it provides a bloodless and immobile operating field. In this method, gas exchange through the heart-lung machine (pump) is performed outside the body with the help of oxygenators, and blood is sent back to the body to perfuse organs. Reactive oxygen species can affect morbidity and mortality during and after cardiac surgery.[13] According to some theories, tissue damage, endotoxemia, and contact of blood with a foreign surface during CPB are the main causes of the systemic inflammatory response, resulting in oxidative stress.[14–16]

In the literature, MDA levels obtained during CPB surgery increased in different periods of application. For example, it is stated that superoxide radical production and MDA level increase within the reperfusion period at the end of ischemia in patients undergoing CPB.[17,18]

Cohen et al.[19] examined the total antioxidant capacity and lipid peroxidation before CPB and at the 1st, 6th, 24th, and 72nd h after CPB. The researchers observed a significant decrease in total antioxidant capacity, and a significant increase in lipid peroxides occurred at the 1st h after the end of CPB, and both parameters reached their normal values at 72nd h.

In another study, it was found that MDA levels were high only in the 1st h of CPB operation; and they returned to their pre-operative levels at 6th, 12th, and 24th h.[20] The results of both studies are similar to our findings in Stage VII (G). Coghlan et al.[21] stated that the significant increase in TBARS level in the systemic circulation occurred at the 2nd and 5th min after the removal of the cross-clamp from the aorta. Starkopf et al.[22] reported that the MDA levels increase 15 min after the beginning of the operation, and the high MDA levels continued in the reperfusion periods after the aortic cross-clamp was removed.

In our study, MDA levels started to increase soon after the circulation connected to the EC system. This increase reached a maximum during the disconnection of circulation from the pump, in which the reperfusion is the main factor. In the post-operative intensive care stage, it was observed to decrease. The significant increase related to the connection of the circulation into the EC pump may be associated with the triggering of inflammation and other concurrent events due to the contact of blood with non-physiological surfaces in the EC system. Its peak at the pump-out stage can be interpreted as a reperfusion-dependent increase of oxidative stress in the heart tissue, which is in accordance with the literature.[20]

Berg et al.[23] found that 8-iso-PGF2α, which they measured as a marker of oxidative stress, increased after the initiation of surgery, but did not increase further during CPB and after aortic cross-clamp removal.

It has been thought that ROS are effective on morbidity and mortality during and after cardiac surgery. The positive results in antioxidant-supported studies confirm this finding.[13] However, no sufficient study has been carried out to explain which antioxidants at what dosages should be used or how to improve the effects of endogenous antioxidants, such as glutathione (GSH).

İşlekel et al.[24] reported a decrease in glutathione peroxidase (GPx), glutathione reductase (GRx) activities, and GSH/GSSG ratio and an increase in SOD activity in similar patients after clamping the aorta. Inal et al.[17,25] reported an increase in SOD and GRx activity, a decrease in catalase activity, and no change in GSH and GPx levels in patients who underwent cardiac surgery, and they gave attention to the development of oxidant damage. Researchers stated that since the degree of oxidative stress is determined by the rate of formation of oxidant molecules and the total potency of antioxidant molecules, the examination of one type of antioxidant molecule may be insufficient to show oxidant stress.

Luyten et al.[26] examined the total antioxidant capacity together with GPx and SOD enzymes at the 10th min of EC, at the end of EC, and after surgery in patients who underwent coronary artery bypass grafting. In these measurements, oxidant parameters increased at the end of the EC, and the antioxidant capacity increased to the highest level at the 10th min of the EC. Carlucci et al.[27] observed a decrease in cellular NAD, GSH levels, and plasma nitrate, nitrite, and GPx levels during the ischemic period in their similar studies. The researchers, who could not find a significant difference in reactive oxygen metabolites, determined the increased ET1 levels 30 min after reperfusion. Kunt et al.[28] observed that the total antioxidant capacity decreased and the oxidative stress index increased from the beginning to after the surgery, respectively. They stated the negative correlation in total antioxidant capacity during surgery.

Different findings and evaluations can be encountered depending on the use of different parameters in the investigation of antioxidant systems.[29,30]

In our study, total sulfhydryl groups, which are indicators of GSH values, were measured. It is noteworthy that patients' RSH levels started to decrease significantly with the connection to the pump, and this decrease reached the highest level in the stage of disconnecting from the pump.

The use of antioxidants may have beneficial effects. Sucu et al.[13] showed that N-acetylcysteine has beneficial effects on CPB patients. On the other hand, it has been stated that the cold effect during surgery negatively affects the antioxidant capacity.[31]

NO is the other parameter measured in CPB surgery. Direct measurement of NO is very difficult under basal conditions because of its very short half-life.[32,33] For this reason, we measured NOx levels.

Increased NO levels during EC have been shown by some authors.[34–36]

Clermont et al.[37] found the increased plasma NO levels at the end of the cross-clamp and during myocardial reperfusion in patients undergoing CPB, but it was not significant. Sharma et al.[38] investigated coronary sinus blood samples in similar pa-

tients before bypass, at 30th min after cross-clamping (ischemia), and 10 min after clamp removal (reperfusion). Reactive nitrogen intermediates increased in the ischemia phase, but a significant increase occurred in the reperfusion phase.

Viaro et al.[39] reported the non-correlated NOx levels and hemoglobin levels, which are a "scavenger" for NO. The investigators concluded that the increase in NO during CPB may not be an indicator of the inflammatory response, due to inducible nitric oxide synthase activation starting 24 h after cytokine increase.

However, it can be expected that the surgical procedures performed in the preparatory phase between opening the thorax and connecting to the pump trigger inflammatory responses. Furthermore, changes that may occur in erythrocytes during the pump phase may affect NO increase.

Fischer et al.[40] examined the erythrocyte eNOS activation in blood samples taken at 0, 20, 40, 60, and 80th min of CPB in patients who underwent coronary artery bypass grafting and valve replacement to explain the mechanism of hypotension that frequently occurs in CPB and hemodialysis. The eNOS activation was significantly increased. Based on these findings, the researchers stated that erythrocytes may contribute to hypotension by releasing NO during CPB and concluded that the mechanical stress created by EC activates the eNOS in the erythrocytes. They suggested that this activation may occur with the transition of eNOS bound to the cytoplasmic membrane into the cytosol.

Cosby et al.[41] stated that hemoglobin in erythrocytes can be circulated in the form of S-nitrosohemoglobin or nitrosylhemoglobin by reversibly binding with nitrite and that hemoglobin can form NO by functioning as nitrite reductase. Kleinbongard et al.[42] demonstrated the activated and functional eNOS in erythrocytes.

Our findings are in line with studies reporting that NOx levels increase in CPB after connecting to the pump. Our NOx levels reached the highest level at the pump-out stage. The NO increase at the beginning of EC may be associated with the activation of eNOS in erythrocytes by the mechanical stress within the pump and the contact of the blood with the foreign surface.[40] NOx levels decreased during the post-operative intensive care period.

However, the values were also higher than those in the pre-operative intensive care and anesthesia stages. It may be expected that the continuous blood flow mechanically stimulates the blood vessel wall at the pump stage. The friction may also activate the eNOS in endothelium cells via protein kinase.[9] Because "Shear stress" is the most important physiological stimulant that causes NO synthesis in vessel endothelium.

We also measured plasma viscosity in seven different stages. In CPB surgery, perfusion is provided by maintaining a suitable hemodilution and temperature based on blood flow.[43–45] Blood viscosity depends on plasma viscosity, hematocrit levels, the deformation and agglomeration of red blood cells, and temperature. Under CPB conditions, the administered cold blood cardioplegia, anticoagulants, or the liquids used in hemodilution may change the blood viscosity.[46] Blood viscosity is affected by a variety of factors, but it is valuable to help perfusion management.

In our study, plasma viscosity decreased with the connection to the pump and increased with the disconnection from the pump. Most likely, this is the result of the denaturation of plasma proteins and the dilutional effect of pump circulation.[47] This situation may be associated with the binding of the proteins to the wall structure during circulation in the pump or the effects of hemodilution in the pump, which is up to 40%.[48] It has not reached pre-operative plasma viscosity levels at the pump-out stage. Therefore, albumin replacement at the pump-out stage may be recommended.

Not comparing viscosity levels with hematocrit levels may be a limitation of our study. However, short-term hematocrit changes due to hemodilution, hemoconcentration, and transfusion can arise during EC. It has been shown that the blood viscosity decreases in hemodilution, but increases in extreme hemodilution, which uses viscogenic plasma expanders. It has been reported that the restoration of blood viscosity is as important as the O_2 carrying capacity in the management of hemorrhagic shock.[49] Furthermore, blood is a non-Newtonian fluid; its viscosity decreases as a result of red blood cell deformation at a high shear rate, and its viscosity increases as a result of red blood cell aggregation at a low shear rate.[50] The decrease that we found in viscosity may be due to red blood cell deformation that occurs during the EC process.

It has been thought that the increase in functional capillary density, which is seen as a response to wall shear stress increase, is provided through NO-mediated vasodilatation under normal physiological conditions. High NO levels measured concurrently with low viscosity in our study contradict the findings available in the literature. However, pathological cases such as SIR, edema, and multiple organ failure occurred after CPB complied with our MDA and NO findings. The changes in plasma viscosity may also play a role as a regulator of inflammation and oxygen metabolism, as they affect shear stress and NO production.

Conclusion

Our results regarding oxidant and antioxidant parameters seem to be comparable with the literature. According to our findings, initiation of EC leads to an increase in MDA and NOx levels and a decrease in RSH and viscosity levels. Our NO findings that are inversely related to plasma viscosity, as well as the complications observed following a CPB, make us believe that endothelial sensitivity can not be protected in EC operations and that this may be the cause of SIRS. Inversely related NO levels with plasma viscosity that we observed following CPB, make us believe that endothelial sensitivity may not be protected in EC operations, and this situation may contribute to the SIRS process that may occur in these patients. These results may reveal the importance of antioxidant and plasma protein supplementation in EC procedures. In this context, protein plasmapheresis of the patient's proteins and GSH as an antioxidant support may be valuable. Further investigation may explain the relationship between oxidant-antioxidant status and viscosity with clinical outcomes.

Disclosures

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Informed Consent: Informed consent was obtained from all participants.

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