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THE RELATIONS OF DIABETES, TRACE ELEMENT, OXIDATIVE STRESS, AND HOMOCYSTEINE IN PATIENTS UNDERGONE OFF-PUMP CORONARY ARTERY BYPASS SURGERY

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ABSTRACT **Background:** The main cause of atherosclerosis is the disruption of trace element biobalance and endothelial damage accompanied by increased oxidative stress.

Methods: In this clinical prospective study, all diabetic patients were randomly selected according to the Euroscore II risk classification. Off-pump coronary bypass surgery was performed in all cases. From the blood samples taken before and after the surgery, zinc and copper, malondialdehyde lipid peroxidase (MDA), superoxide dismutase (SOD), total oxidant/antioxidant capacity (TOS/TAC), nitric oxide (NO), HbA1c and homocysteine levels were analyzed.

Results: Early postoperative processes were significantly longer in the diabetic group. Trace elements did not change after surgery. TOS, NO, and SOD activities were significantly increased in all groups compared with the control group. NO levels significantly increased in the T1DM group compared with the control group. ($p < 0.05$). There was a positive correlation between HbA1c and homocysteine, especially in T1DM ($p < 0.05$).

Conclusion: Zn/Cu and SOD levels were not affected by the operation in patients from the same risk group, and the hospital stay was longer in the diabetic group. NO, and MDA levels increased after surgery due to inflammatory stress, but homocysteine levels did not change except in cases requiring a long hospital stay.

KEYWORDS : Atherosclerosis, Off-pump Bypass Grafting Surgery, Diabetes mellitus, Copper/Zinc, Oxidative stress, Homocysteine

INTRODUCTION

Atherosclerosis and related vascular diseases are still the leading causes of death worldwide. Mozarfan et al 2016 [1]. For this reason, combating atherosclerosis plays an important role in protecting public health. Diabetes is one of the most important risk factors for atherosclerosis and an important component of endothelial damage in its formation mechanism. Diabetes can cause oxidative stress through different mechanisms that are not yet fully understood. Gleissner CA et al 2007 [2] It is known that inflammation caused by endothelial damage mainly accompanied by hyperglycemia accelerates atherosclerosis [3-7]. Several studies have reported that zinc and copper are necessary for the removal of free reactive species that occur naturally during metabolism and for the protection of endothelial integrity [8-10]. Francisco Andujar Vera F et al 2020 [11] determined by proteomic analysis that superoxide dismutase (SOD), was effective in femoral artery calcification in T2DM, and required Zn and Cu as coenzymes. Several studies have reported that low trace element levels after coronary bypass surgery are important factors in early postoperative morbidity [12-15].

When reactive oxygen and nitrogen species, which are products of cellular metabolism, are not controlled by protective systems, chain reactions that damage cells begin. Mitaka C et al 2007 [16]. Nitric oxide, a versatile biological molecule, is also a protective molecule in inflammation and thrombi. Endothelial cells perform their NO-related functions by directing the nitric oxide synthase (eNOS) enzyme derived from the endothelium [17-19]. Although there is endothelial damage due to various reasons, diabetes and especially uncontrolled diabetes with HbA1c ≥ 7 stands out as the major factor that triggers the damage in atherosclerosis, Gleissner CA et al 2007 [2], Victorinova et al 2009 [20]. In the process of atherosclerosis formation, inflammation and thrombotic processes coexist simultaneously and interactively. Alturfan EE et al [4] demonstrated that the inflammatory process in atherosclerosis is parallel to thrombotic processes. Several studies have reported that tissue factors play a key role between inflammation and coagulation in atherosclerosis [5].

In a statistical study conducted between 1992-2018, a strong relationship between copper-bearing ceruloplasmin and coronary heart disease (CHD) was reported by Di Nicolantonia JJ et al 2018 [9]. de Larriva A P et al 2020 [10] emphasized the importance of copper in the onset of cardiovascular diseases, among many studies on the relationship between atherosclerosis and trace elements. Several studies have examined the role of zinc and copper in the antioxidant defense system and anti-inflammatory agents, and it is also an important regulator of gene expression [21-24]. Prasad and Bao [25] reported that high Cu values in serum may be related to inflammation in T1DMs.

After coronary bypass, reactive oxygen species based on reperfused myocardial cells and related reperfusion damage may develop more or less. With the systemic effects of these oxidative stress factors, in on-pump coronary bypass cases, the domino effect formed by the contact effect of the heart-lung pump on the polymer set increases the possible systemic effect and activates the inflammatory and coagulation cascades, and this effect, especially the vital end of the nonpulsatile heart-lung pump. Previous studies have shown that this effect, especially the nonpulsatile heart-lung pump, can cause various degrees of damage to vital end-organs. [26]

Currently, coronary bypass is performed with (on pump) or without (off-pump, OPCAB) cardiopulmonary bypass. It is known that oxidative stress increases due to ischemia-reperfusion that occurs when coronary artery bypass grafting (CABG) is performed with an on-pump. This study aimed to evaluate the association of serum zinc, copper, and oxidative stress in atherosclerotic patients with and without diabetes before and after OPCAB surgery.

PATİENTS AND METHODS

Patient Selection

From October 2018 to January 2020, 100 consecutive elective patients undergoing first-time coronary bypass surgery with the OPCAB technique because of severe atherosclerosis were randomized according to Euroscore II risk stratification – as a moderate-risk category (Euroscore mean±SD: 4.31±0.65) enrolled.

All experimental protocols were approved by the Ethics Committee of Istanbul Medipol University, number and date:10840098-604.01.01-E.47329/25.10.18, and approved by the Ethics Committee of Koç University Hospital, number, and date: EMA/hd/54/2019/07 informed consent was obtained from each patient.

All methods were performed according to the relevant guidelines and regulations of the Declaration of Helsinki.

Actual study start date: 30/10/2018

Actual primary completion date: 01/01/2020

Actual completion date: 01/01/2020

None of the cases had heart valve disease or preoperatively detected atrial fibrillation attacks. All of the patients were between the ages of 40-65, 35/100 of whom were female, and the BSI was 1.77±0.055. Beta-blocker and statin therapy were given to all patients preoperatively in preparation for the beating heart. None of the patients had a preoperative problem that might have end-organ damage (renal, neurological, hepatic, or respiratory). None of the patients in our study gave inotropic agents either preoperatively or postoperatively. This is one of the factors that reduces oxidative stress.

Surgical Techniques

In our study, patients with advanced coronary atherosclerotic patients (N = 100, all in the same risk category, Euroscore II) were included. Off-pump coronary bypass was performed in all cases with the same surgical technique/protocol and the same surgical team.

Left internal thoracic artery (ITA, LIMA) and radial artery grafts were prepared after appropriate heparinization (100 IU/kg and ACT> 300) under general anesthesia and median sternotomy. After stabilization provided by retraction stitches in the heart, coronary bypass surgery was performed in the beating heart with the aortic "no-touch" technique, that is, without placing a partial or full clamp on the aorta. Coronary bypass was performed entirely with arterial grafts. Bypass was performed with composite grafts created by anastomosing the radial artery on the LIMA in "T" or "inverted Y" -shaped configurations. The prepared radial artery graft was kept in a solution with heparin and a calcium-channel blocker until use during surgery. The solutions prepared for the patient and the graft during the operation were normothermic. Neither perioperative nor postoperative inotropic support was used in any of the patients included in the study.

Blood Sample Collection And Analysis

After obtaining consent forms from selected reference individuals, blood samples were collected between 08:00-12:00 in the morning at the end of 8-12 hours of fasting. Blood samples were taken from the antecubital vein in a sitting position into 8-milliliter vacuum gel red-capped tubes. The blood samples were centrifuged at 3000 rpm for 10 minutes using a NUVE (NF-800R) brand centrifuge, and serum samples were separated. The separated serum samples were taken into Eppendorf tubes and stored at minus 80°C until analysis.

Blood samples were collected before and 3 days after CABG.

One hundred atherosclerotic CABG patients were included as nondiabetic (n = 50) and diabetic patients (n = 50). The diabetic group was divided into two groups: Type 1 (T1DM, n=25) and Type 2 (T2DM, n=25).

Zn-Cu levels

Serum Zn and Cu concentrations were measured with commercially available measurement kits (Rel Assay Diagnostics, Gaziantep, Turkey) using a fully automatic photometric method (Abbott ARCHITECT ci16200 clinical chemistry analyzer).

HbA1c, CRP And Homocysteine Levels

HbA1c levels were measured by turbidimetric inhibition of hemolyzed whole blood, and CRP levels were measured by the particle surface expanded immunoturbidimetric test method using the Roche Cobas 6000 device (Roche, Germany). Homocysteine levels were measured by the immunoassay method, using an Immulite 2000 device (Siemens, Germany).

SOD Activity

A method based on the effect of photooxidation of the o-dianicid of SOD sensitized with riboflavin was used. The absorbance of the product was measured at 460 nm in a spectrophotometer. Net absorbance was calculated by measuring the absorbance values at 0 and 8 minutes after illumination using 20 W fluorescence lambs. The results are expressed as U/min g protein (27).

Lipid Peroxidation

LPO's MDA products were identified as thiobarbituric acid reactive substances (28). LPO was expressed in MDA equivalents using an extinction coefficient of 1.56 × 105/M-1/cm-1.

Total Antioxidant Capacity And Total Oxidant Status

TAC and TOS serum concentrations were determined colorimetrically in serum (29,30).The TAC method based on antioxidants neutralizes existing ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (Sigma-Aldrich, Taufkirchen, Germany) radicals in serum. Absorbance is measured at 658 nm. In the TOS method, Fe2SO4 dissolves in water and releases Fe2+. Oxidants oxidize from Fe2+ to Fe3+, the reagent X-orange (Sigma-Aldrich Taufkirchen, Germany) gives a colored complex, and the absorbance is measured at 658 nm.

Nitric Oxide

Nitric oxide (NO) was determined by the method of Miranda et al 2001 [31] based on the reduction of nitrate to nitrite with vanadium-(III) chloride. Nitrite and sulfonyl amide react with N-(1-naphthyl) ethylenediamine dihydrochloride to form a complex diazonium compound. The complex was measured with a spectrophotometer at 540 nm.

Data Analysis

For continuous variables, means and standard deviations (SD) and categorical variables N (%) were expressed for all demographic and clinical characteristics and serum parameter levels by the group. The Kolmogorov-Smirnov test was used to test normality. Data were analyzed using IBM SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). ANOVA and the Kruskal Wallis test were used to examine differences between groups for continuous variables. The chi-square test of independence was used to test group differences for categorical variables. Partial Spearman rank correlation analysis was used to examine associations between variables.

P<0.05 was considered significant.

Logistic regression was performed to ascertain the effects of EuroScore, ejection fraction, hospital time, intubation time, and difference in pre- and postmeasurements of SOD, TOS, MDA, ZN, CU, and NO on the likelihood that participants had diabetes. ICU time and differences in TAC were excluded because of the high correlation of other variables. The logistic regression model was statistically significant, $\chi 2(4) = 79.792$, $p < .0001$.

The model explained 73.0% (Nagelkerke R2) of the variance in heart disease and correctly classified 91.0% of cases. Diabetics length of stay was 2.531 (95% CI: 1.14;5.621) and intubation time was 2.472 (95% CI: 1.051;5.813) time higher than nondiabetic patients. An increasing difference in NO was associated with an increased likelihood of exhibiting diabetes, but an increasing difference in MDA was associated with a reduction in the likelihood of exhibiting diabetes. (Table 3)

RESULTS

Alteration Of Demographic And Clinical Values Of Study Subjects

There were no significant differences in sex, smoking, BMI, euro score scores, ejection fraction, or total drainage (p>0.05). [Table 1].

Early postoperative processes of diabetic patients, intubation time, ICU, and hospital stay were significantly higher than those of nondiabetic patients.

In the diabetic group, in comparison between the T1DM and T2DM subgroups, the duration of intubation, ICU stay, and hospital stay were significantly longer in the T1DM group [Table 1].

The early postoperative processes (intubation, ICU, and hospital stay) of the female patients in the diabetic group in our series were found to be significantly longer than those of the nondiabetic patients (in the same group according to age and risk stratification category, EuroScore II, respectively; p= 0.025, p= 0.012, p= 0.021).

There was no significant difference between those in menopause, but since the number is small, it will be considered a study limitation and will be investigated in further studies with a large number of samples.

Alteration Of Plasma Zn And Cu Concentration

Plasma Zn concentration compared to the control group It was found to be significantly higher in the nondiabetic atherosclerotic group compared to the diabetic group.

There were no significant differences in Zn concentration before and 3 days after OPCAB.

Although the serum Cu concentration was significantly lower in nondiabetic atherosclerotic patients than in the control group, there was no change before the operation or on the 3rd day of the operation [Table 2, Figure 1A,1B]. A significant correlation was found between Zn and Cu only between T2DM.

Alteration Of Plasma HbA1c, CRP, And Homocysteine

Plasma HbA1c and homocysteine values were evaluated between groups. In the nondiabetic atherosclerotic group, there was a positive correlation between HbA1c and homocysteine.

HbA1c levels in T1DM patients were significantly higher than those in T2DM patients and nondiabetic patients.

The HbA1c level was found to be significantly higher in the diabetic group than in the nondiabetic group, and the early postoperative processes were also longer [Table 1]

Although CRP values were higher in T1DM patients before the operation than in non-DM and T2DM patients, there was no significant difference between the groups due to their standard deviations. No significant difference was found between all groups in the values after the operation [Table 2]. In addition, there was no significant correlation between CRP values and HbA1c, homocysteine, Zn, Cu values.

The homocysteine value in the diabetic group was higher in T1DM than in T2DM [Table 1].

Alteration Of Plasma NO And MDA

Before the operation, nitric oxide (NO) values, according to the control, were lower in the nondiabetic group and higher in the T1DM group. T2DM did not differ compared to the control [Table 2].

A postoperative change in NO values was seen as a more significant decrease in T1DM than in T2DM. Although the NO values in T2DM showed a significant decrease compared to the control, the change before and after the operation was less than that in the T1DM group [Table 2, Fig. 2].

The MDA values we measured as an indicator of lipid peroxidation were found to be significantly higher in all three groups compared to the control. After the operation, a significant increase was observed in both subgroups of the diabetic atherosclerotic group (T1DM and T2DM) compared to the nondiabetic group [Table 2, Figure 2].

Alteration Of Plasma SOD Activity And Oxidant/Antioxidant Status (TOS/TAS)

Before the operation, SOD values were significantly higher in the NonDM, T1DM, and T2DM groups than in the control group. There was no significant change in any of the three groups after the operation. Total antioxidant status (TAS) was significantly lower in the atherosclerotic group, especially in the T2DM subgroup, than in the preoperative control group. Total oxidant (TOS) values were found to be higher in the atherosclerotic group, especially in the T1DM group, than in the control group [Table 2, Figure 3].

There was no significant change in any of the three groups after the operation.

In the nondiabetic atherosclerotic group, TAS balance negatively correlated with preoperative SOD. Advanced significant positive correlation between preoperative TOS and TAS. This may be a significant clinical predictor of preoperative TAS/TOS assessment in the nondiabetic atherosclerotic group. The preoperative NO value was significantly negatively correlated with the preoperative TAS/TOS values. Preoperative Cu values were negatively correlated with preoperative TOS values. The Zn values are positively correlated with TAS and TOS and negatively correlated with NO values.

DISCUSSION

The key or inducing mechanism in the formation of atherosclerosis is endothelial injury that occurs for various reasons. Diabetes, in particular uncontrolled diabetes, comes to the front as the major factor inducing this injury in cases where HbA1c is ≥7 [3]. Various studies have underscored that oxidative stress accompanied by impaired trace element biobalance is a considerable cause of diabetes that accompanies or increases its potential to cause endothelial injury [6-11].

Reactive oxygen species (ROS) that occur naturally in oxygenated respiration can damage the antioxidant system in various diseases. In addition to many other effects, Zn and Cu are significant components of the superoxide dismutase of SOD and have a crucial role in removing ROS. Various studies have underscored that oxidative stress is important in the pathogenesis of both diabetes types. Deterioration of antioxidant balance due to varying reasons is among the causes of diabetes and atherosclerosis. SOD, catalase, glutathione reductase, glutathione peroxidase, lipid peroxidation, nitrite concentration, nonenzymatically glycosylated proteins, and hyperglycemia are among the biomarkers of oxidative stress in diabetes. In atherosclerosis, which is a multifactorial disease characterized by endothelial dysfunction, increased HbA1c values together with elevated blood glucose levels can alter DNA damage, protein dysfunction, and signal transmission. Elevated blood glucose levels and decreased NADPH levels also facilitate the progression to cellular redox [6-9] Ultimately, individuals who have been diagnosed with diabetes and developed advanced endothelial dysfunction may die from macrovascular coronary artery disease while struggling with the morbidity of microvascular complications.

The present research is a prospective, clinical study, which was conducted in a randomized large series at the same risk level, in which off-pump coronary bypass (OPCAB) surgery was performed without the use of a heart-lung machine (cardiopulmonary bypass machine) for biochemical and clinical evaluations.

Emad ALJ et al [12] stated their limitations in their meta-analysis; only 2 of the 11 studies were randomized controlled clinical studies. One of the important features of our study is that the analyses were the largest homogenized, single-center series, selected from among the patients who underwent OPCAB only, and without possible/potential dilutional effects of the heart-lung pump or possible additional inflammatory and thrombotic processes.

In our clinic, a total of 100 consecutive patients with critical atherosclerosis included in our study were in the same risk category (EuroScore II), randomized in equal numbers as two groups of diabetic and nondiabetic patients who underwent OPCAB surgery with the same surgical method and team. Analyses were performed on preoperative and postoperative 3rd-day blood samples taken from the patients who were included in the study. Of trace elements, zinc and copper, oxidative stress parameters (SOD, MDA, TAS, TOS, nitric oxide, CRP, HbA1c) and homocysteine results were analyzed, and all parameters were compared with early postoperative clinical findings (duration of intubation, intensive care unit and hospital stay durations). The diabetic group was examined in 2 subgroups, insulin-dependent (T1DM) and noninsulin-dependent (T2DM), and relevant correlations were made. All analyses in both the nondiabetic atherosclerotic group and the diabetic-atherosclerotic group were compared with the age and sex-matched nonatherosclerotic volunteer-healthy control group.

In our series, of the early postoperative processes after OPCAB, the intubation, ICU, and hospital stay durations were determined to be significantly higher among the diabetic group ($p<0.001$, Table 1). Upon examining the laboratory parameters, it was determined that the preoperative Zn value was significantly higher among patients with nondiabetic atherosclerosis than among the diabetic group, while the Zn value in atherosclerosis patients with T1DM and T2DM was not different from that in the control group, and no significant change was found between Zn values before and after undergoing OPCAB ($p>0.05$). [Table 2, Figure 1A]

Oxidative stress occurs in patients undergoing coronary artery bypass operations. Following coronary bypass surgery, reactive oxygen radicals based on reperfused myocardial cells and related reperfusion injury may develop to some degree. Together with the systemic effects of these oxidative stress factors, the contact effect of the heart-lung pump on the polymer set of the heart-lung pump increases the potential systemic effect in patients on-pump CABG cases by activating the inflammatory and coagulation cascades with a domino effect. The oxidant-antioxidant balance deteriorates due to oxidative stress pathways induced by enzymatic and nonenzyme mechanisms.

Gonenc et al. [13]. As stated in the OPCAB series of 30 cases, SOD and MDA levels were found to be higher in the on-pump bypass arm, and they attributed this outcome to the inflammatory process induced by contact with the extracorporeal surfaces of polymer tubing sets used for cardiopulmonary bypass. Researchers highlighted that antioxidant balance is impacted less by OPCAB. Similarly, in all atherosclerotic groups in our larger series, the preoperative SOD value was significantly higher than that in the control group ($P<0.001$), and no significant change was noted in the postoperative period.

In the diabetic atherosclerotic group, the determination of a significant negative correlation between Zn and MDA indicates that exogenous Zn supplementation could be favorable for the uncontrolled diabetic patient group. A significant negative correlation was determined between SOD values and TAS balance in the preoperative nondiabetic atherosclerotic group. In the diabetic atherosclerotic group, a significant negative correlation was determined between SOD and the duration of intubation and ICU stay from early postoperative processes. This suggests that SOD and TAS balance could be significant clinical predictors. Moreover, NO values, which were found to be significantly higher in the atherosclerotic group, were negatively correlated with the TAS/TOS balance. A highly significant positive correlation was found between the preoperative NO level and the intubation and ICU stay durations from the early postoperative processes.

On the 3rd postoperative day after OPCAB, no significant change was detected in SOD values, total antioxidant (TAS), or total oxidant status (TOS) ($p>0.05$). Preoperative TAS values were found to be significantly lower in the atherosclerotic group, particularly in the T2DM subgroup. On the other hand, TOS values were determined to be higher in the diabetic atherosclerotic group, especially in T1DM, than in the control group.

Many studies have demonstrated the detrimental and protective effects of nitric oxide.

A naturally occurring analog of L-arginine asymmetric dimethylarginine (ADMA) is a competitive inhibitor of NOS. The presence of endogenous NOS inhibitors in vivo has been identified as one of the mechanisms associated with decreased NO production. Plasma levels of ADMA are elevated in patients with hypercholesterolemia and atherosclerotic vascular disease. In our study, NO levels were found to be high, which can be explained by the low LDL-C levels in our patients under statin therapy, as seen in Table 1, due to the possible statin effect. [16-19].

Contrary to what was thought in the past, it has been reported that CPB will contribute to endothelial damage, although the systemic inflammatory response is not entirely dependent on the pump effect and is attributed to surgical stress. In our series, the fact that thanks to not using CPB and not placing partial or full aortic clamps, general ischemia did not occur, and it remained only at a local level due to the occluded coronary artery, or that stabilizers, which could cause myocardial cell damage due to compression, were not used, and that inotropic agents were not used in the perioperative and postoperative early period, can be considered favorable protective contributions to endothelial function. Successful early postoperative processes with determined high NO values seem to prove this finding.

In their study, Mitaka C et al [16] considered urinary NO excretion as an indicator of endogenous NO production following CPB, and they found in their series that the highest endogenous NO production was in patients who underwent OPCAB. In this study, they found a positive correlation between urinary nitrate excretion and the postoperative cardiac index and a negative correlation between systemic vascular resistance. The favorable effect of endogenous NO production is consistent with the results we obtained in our study, and as all patients in our series underwent off-pump coronary bypass, they did not require either perioperative or postoperative inotropic support, and they had a positive effect on early postoperative processes, including early extubation in terms of respiratory function, positive effect on cardiac functions, and durations of ICU and hospital stay.

In their study, Viktorinova A et al. [20] measured zinc and copper values in T2DM and healthy individuals and determined a negative correlation between zinc and copper. The researchers found a similar correlation with glycated hemoglobin and considered that the imbalance that occurs in trace elements would worsen diabetic complications.

In our study, Zn was determined to be significantly higher in the nondiabetic atherosclerotic group than in the diabetic group. All early postoperative processes were shorter in this group. However, no significant correlation was found between Zn and Cu levels and HbA1c in any group.

Noshin TF et al. [23] found a significant increase in serum MDA levels compared to the control group in their study and determined a significant decrease in the Zn values of the same patients, whereas the Cu values of the patients increased. In our study, the MDA values resulting from lipid peroxidation were significantly higher in all atherosclerotic groups than in the nonatherosclerotic control group. We found a significant increase in postoperative MDA values in both diabetic subgroups compared to the nondiabetic group [Table 2, Figure 2]. Squitti R et al. [24] examined copper values in T1DM diabetic and control groups and suggested that high copper values were associated with inflammation.

In the study of Prasad AS et al. [25], which was conducted in 2010 to investigate the atheroprotective effect of zinc, 40 healthy individuals aged 56-83 were included in the study and randomly divided into two groups. Upon comparing the group that received 45 mg/day zinc for 6 months, with the group that received placebo, it was revealed that plasma zinc concentration increased and inflammatory parameters (hsCRP, IL-6, macrophage chemoattractant protein) decreased after 6 months in those who received zinc. Prolongation of the diabetic period, perhaps homocysteine, an amino acid containing HbA1c and trace element, sulfur, is required for intravascular metabolism.

Souza ABD et al [32] reported lower oxidative stress results in the OPCAB group than in the on-pump group, although the number of cases was small.

Although the difference between on- and off-pump CABG is revealed partially or relatively less in these and similar studies, in our study, two major factors that may cause oxidative stress in patients who underwent OPCAB were not used; these are the ischemic release of the aorta (partial or full aortic clamping) and by our OPCAB surgical protocol, patients are slowed down in heart rate and consequently their metabolism with preoperative beta-blockers, so inotropic agents are not used both during the surgery and in the early postoperative period, and it can be thought that this reduces the perioperative oxidative stress. Nonetheless, it can be considered that the difference seen in uncontrolled diabetic patients increases the stress at the cellular level by adversely affecting the atherosclerotic background with different inflammatory mechanisms.

Similar to our results, Yaghoubi A et al. [33] reported that oxidative stress markers were increased in on-pump CABG, whereas oxidative stress was less common in cases of off-pump CABG, and consequently, they observed shorter durations of intensive care and hospital stays. In this study, which was conducted with 100 patients, the off-pump technique was used in half of the patients. In this study, unlike our study, diabetes was not investigated and was even considered one of the exclusion criteria. Although Emad AIJ. et al [34] stated in their study that "OPCAB may have similar or slightly reduced long-term survival compared with on-pump CABG", no significant difference was found in the meta-analysis.

The technique used by Fukurawa N et al. [35] in their study corroborates the positive early postoperative outcomes following OPCAB, which we emphasized in our study. We found that the uncontrolled diabetic group with higher HbA1c values had longer ICU and hospital stays. Researchers have investigated the effects of off- and on-pump CABG on mortality and readmission, although hospital readmission in 5 years was more common among off-pump CABG cases.

In a recent study, Rufa MLet al. [36] reported lower morbidity and mortality in the off-pump arm in their study, and they emphasized that off-pump CABG could be performed with lower morbidity and mortality in highly experienced reference centers [34-37]. Our healthcare center is one of the reference centers where off-pump coronary bypass is performed in our country, and the technique is routinely used as a priority treatment option for almost every patient.

Increased thrombogenicity, oxidative stress status, and endothelial dysfunction are observed in high homocysteine values. High total homocysteine levels are characterized by increased aortic rigidity and insulin resistance in Japanese patients with type 2 diabetes. Although these results indicate that hyperhomocysteinemia could be associated with atherosclerosis, the molecular mechanisms that would illuminate this relationship are not yet fully known [38-42]. In our study, a significant positive correlation was determined between homocysteine levels and ICU length of stay among the diabetic atherosclerotic group.

CONCLUSION

In this study, off-pump coronary bypass surgery was performed in all selected patients in the same risk group, and Zn/Cu and SOD levels were not affected by the operation. Since different hyperglycemia-related mechanisms may promote diabetic atherosclerosis, we think that as a result of our study, keeping the blood sugar level at reference values below in advanced atherosclerotic diabetic patients who will undergo off-pump coronary bypass surgery will have a positive effect on the clinic. The diabetic group had long hospital stays as a result of impaired antioxidant biobalance. NO, and MDA levels increased after early surgery due to inflammatory stress, but homocysteine levels did not change except in diabetic patients requiring a long hospital stay. The 1- and 5-year clinical results of the cases should be analyzed with multicenter, larger studies to be conducted.

Study Limitations

Since OPCAB coronary bypass is used in every case that is routinely appropriate in our clinic, the limitation presented in our study can be eliminated by comparing the results with randomized large series using a heart-lung machine.

Conflict Of Interest:

There is no conflict of interest in our study.

Table 1. Demographic Clinical And Operative Characteristics

	NonDM (n=50)	T1DM (n=25)	T2DM (n=25)	Test Statistics	P value	s/ns
Age (years)	54.96± 7.52	54.28±7.28	57.44±4.73	F (2.55)=2.3	0.110	ns
Gender-Female (%)[b]	18(56.25%)	10(66.66%)	8(47.06%)	X^2=0.347	0.841	ns
Body mass index (BMI)[a]	1.77±0.52	1.77±0.71	1.77±0.45	X^2=0.137	0.934	ns
Smoking [b]	19 (38%)	4(16%)	9(36%)	X^2=3.952	0.139	ns
Homosistein(µmol/L)[a]	13.23±4.16	14.70±3.93	11.76±3.91▲	X^2=1.104	0.032	s
HbA1c(%)[a]	4.08±0.22	8.02±1.72*	5.60±0.55*▲	X^2=86.824	0.001	s
Euroscore	4.20±0.45	4.52±0.87	4.32±0.69	X^2=1.824	0.402	ns
Ejection fraction[a]	54.51±2.95	56.16±4.70	55.96±3.82	X^2=0.886	0.642	ns
Hospital time(days)[a]	3.92±0.87	9.88±1.56*	4.92±1.22*	X^2=65.204	<0.001	s
ICU time (time/day)[b]	1.08±0.54	1.93±0.51*	1.21±0.21▲	X^2= 75.00	<0.001	s
Entube time (hour) [a]	2.42±0.81	8.80±1.84*	2.96±0.97▲	F =28.042	<0.001	s
Total drenage (ml) [a]	417.80±162.68	490.20±221.63	462.80±169.37	F(2;97)=1.46	0.236	ns

Data are expressed as mean ± standard deviation. Gender. smoking and ICU time data are given n(%). *p<0.001: Significantly different from the NonDM group, ▲p<0.001: Significantly different from the T1DM group.
ns=non-significant mean comparison.
s= significant mean comparison.
a= Pearson Chi-Square
b= Kruskall-Wallis

Table 2. C- Reactive Protein And Serum Oxidant-Antioxidant Levels.

	Control		NonDM (n=50)	T1DM (n=25)	T2DM (n=25)	Test Statistics	P value	s/ns
Zinc (µg/dl)	87.18±6.58	preOP	103.27±15.61	80.28±13.94	86.29±14.60	16.07	0.001 [b]	s
		postOP	104.78±18.76	81.90±13.11	86.60±15.06			
		Δpost-pre	1.50±20.05	1.62±7.46	0.30±6.65		0.339	ns
Copper (µg/dl)	107.3±8.78	preOP	83.37±13.42	88.03±9.87	85.20±13.47	37.41	<0.001 [b]	s
		postOP	81.86±12.41	87.42±12.31	84.10±13.74			
		Δpost-pre	-1.50±13.27	-0.61±8.48	-1.10±3.72		0.603	ns
C-reactive protein (mg/L)	Ref. value <5	preOP	1.36±0.91	3.52±3.75	1.82±1.98			
		postOP	2.62±1.45	3.71±2.80	2.35±1.41			
		Δpost-pre	1.26±1.44	0.19±3.82	0.81±2.27		0.042	ns
SOD (U/g protein)	39.86±9.68	preOP	68.63±11.77	67.44±12.80	74.41±11.09	50.31	<0.001 [b]	s
		postOP	64.32±15.92	67.30±11.28	76.08±11.35			
		Δpost-pre	-4.31±20.11	-0.13±11.22	1.67±13.21		0.168	ns
TAC (mmol/L Trolox eq.)	0.82±0.07	preOP	0.76±0.14	0.75±0.12	0.69±0.17	9.06	<0.001 [b]	s
		postOP	0.79±0.12	0.71±0.16	0.70±0.18			
		Δpost-pre	0.03±0.12	-0.03±0.12	0.01±0.17		0.099	ns
TOS (µmol/L H202)	22.01±3.51	preOP	25.06±1.87	31.29±2.74	23.58±3.56	54.23	<0.001 [b]	
		postOP	23.75±3.69	30.31±2.50	22.70±3.18			
		Δpost-pre	-1.31±3.08	-0.98±1.38	-0.88±2.90		0.124	ns
NO (µmol/L)	15.25±2.23	preOP	10.94±1.17	19.45±2.91	12.8±81.45	81.39	<0.001 [b]	s
		postOP	10.31±0.76	17.50±3.29	12.97±1.27			
		Δpost-pre	-0.62±1.12	-1.94±3.70	0.09±1.30▲		0.011	s
MDA (nmol/ml)	1.22±0.34	preOP	2.85±0.47	3.67±0.46	2.80±0.47	77.2	<0.001 [b]	s
		postOP	3.08±0.57	3.57±0.57	2.31±0.52			
		Δpost-pre	0.22±0.66	-0.10±0.65*	-0.48±0.70*		<0.001 [b]	s

Data are expressed as mean ± standard deviation. ▪ $p<0.05$: Significantly different from the Control group *$p<0.05$: Significantly different from the NonDM group, ▲$p<0.05$: Significantly different from the T1DM group.
ns=non-significant mean comparison.
s= significant mean comparison.
a= Pearson Chi-Square
b= Kruskall-Wallis

Table 3: Logistic Regression Analysis of Parameters.

Logistic Regression Analysis									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Euroscore	,202	,594	,116	1	,734	1,224	,382	3,918
	Ejectionfraction	,043	,041	1,122	1	,290	1,044	,964	1,131
	Hospitaltimedays	,929	,407	5,207	1	,022	2,531	1,140	5,621
	Entubetime hour	,905	,436	4,300	1	,038	2,472	1,051	5,813
	Δsod	,002	,020	,008	1	,930	1,002	,964	1,041
	Δtos	,036	,138	,069	1	,792	1,037	,791	1,360
	Δmd	-1,919	,652	8,656	1	,003	,147	,041	,527
	Δzn	,032	,025	1,652	1	,199	1,032	,984	1,083
	Δcu	-,009	,041	,046	1	,830	,991	,914	1,075
	Δno	,574	,288	3,971	1	,046	1,775	1,009	3,122
	Constant	-10,444	3,919	7,103	1	,008	,000		

a. Variable(s) entered on step 1: Euroscore, Ejectionfraction, Hospitaltimedays, Intubation/time(h), Δsod, Δtos, Δmda, Δzn, Δcu, Δno : Δ: difference of pre- and post-op values, sod: superoxide dismutase, tos: total oxidant status, mda: malonedialdehyde, zn: zinc, cu: copper, no: nitric oxide.

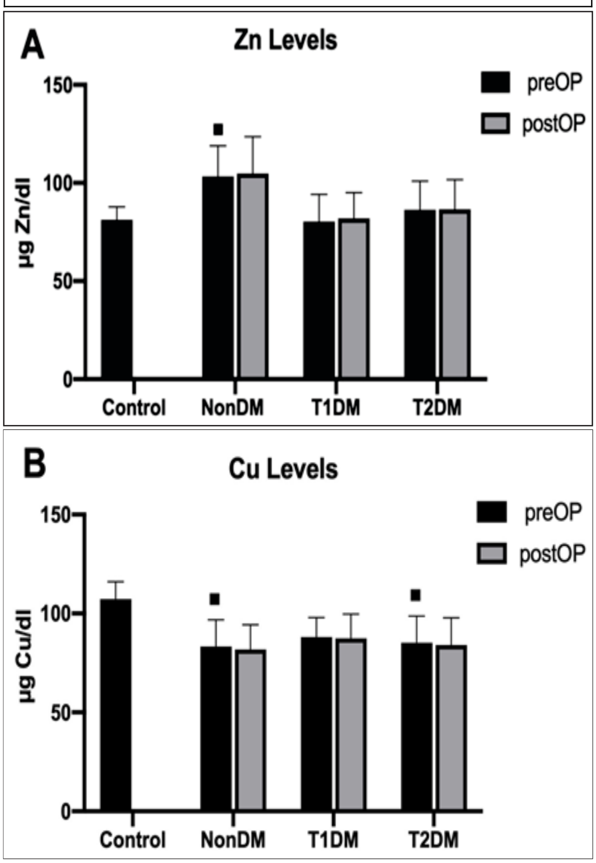

Figure 1. (A)Zn levels (µgZn/dl) of control and the other groups.(B) Zn levels (µgCu/dl) of control and other groups.

Values are given as mean±standart deviation. ▪ $p<0.05$: Significantly different from the Control group *$p<0.05$: Significantly different from the NonDM group, ▲$p<0.05$: Significantly different from the T1DM group.

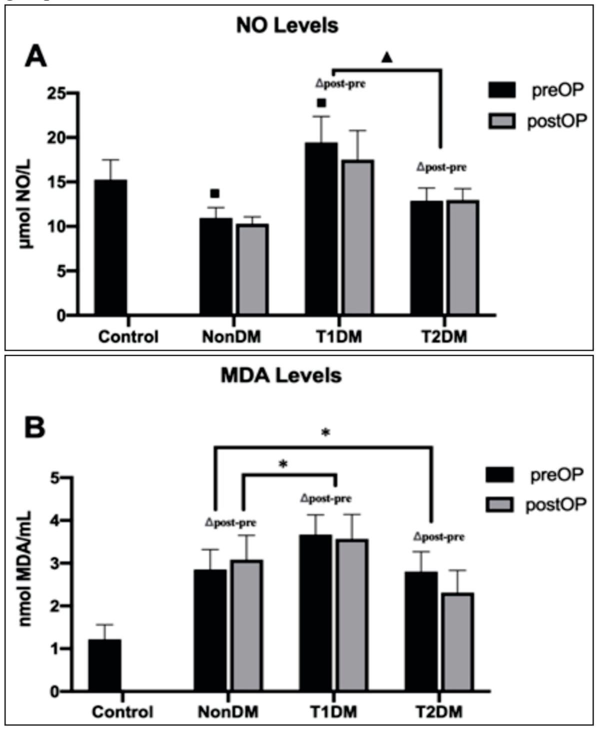

Figure 2. (A)Lipid peroxidation (LPO) levels (nmol MDA/gr) of control and the other groups.(B) Nitric oxide (NO) levels (µmol NO/mg protein) of control and other groups.

Values are given as mean±standart deviation. ▪ $p<0.05$: Significantly different from the Control group *$p<0.05$: Significantly different from the NonDM group, ▲$p<0.05$: Significantly different from the T1DM group.

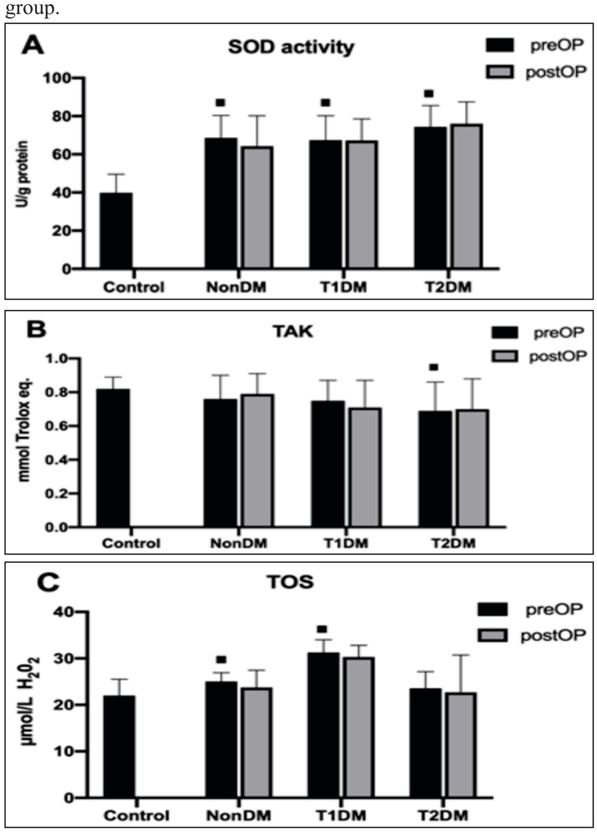

Figure 3. (A)SOD activity (U/g protein) of control and the other groups.(B) TAK levels (mmol Trolox eq.) of control and other groups. (C) TOS levels (µmol/L H2O2) of control and other groups.

Values are given as mean±standart deviation. ■ $p<0.05$: Significantly different from the Control group *$p<0.05$: Significantly different from the NonDM group, ▲$p<0.05$: Significantly different from the T1DM group.

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