

# Investigation of the Effects of Endothelial Protection Solution on Oxidative Stress in Saphenous Vein Endothelium in Diabetic Patients undergoing Coronary Bypass

Bypass Uygulanan Diyabetik Hastalarda Endotel Koruma Solüsyonunun Safen Ven Endotelinde Oksidatif Stres Üzerine Etkilerinin İncelenmesi

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## ÖZET

**AMAÇ:** Diyabetik koroner arter hastalarının baypaslarında kullanılan safen ven greftleri normal hasta popülasyonundan daha erken tıkanır. Bu çalışmada, diyabetik hastalarda operasyon sırasında alınan safen ven greftlerinin oksidatif hasara karşı korunmasında endotel koruma solüsyonunun etkileri araştırıldı.

**GEREÇ VE YÖNTEM:** Hastanemizde 2021-2022 yılları arasında koroner bypass ameliyatı olan 50 diyabet hastasının operasyonu sırasında alınan safen ven greftlerinin kalan kısımları üç parçaya bölündü. Her parça salin (grup 1), heparinize otolog kan (grup 2) ve endotel koruma solüsyonu (grup 3) olmak üzere farklı bir solüsyona yerleştirildi. Çözeltilerde 4 saat bekletildikten sonra hücrel ve DNA oksidatif stres belirteçleri araştırıldı.

**BULGULAR:** Toplam antioksidan kapasite (TAC) açısından gruplar arasında anlamlı fark yoktu ( $p>0.05$ ). Toplam oksidatif durum (TOS) seviyeleri, endotel solüsyonu olmayan grup 1 ve 2'de ( $1,57\pm 0,46$ 'ya karşı  $1,55\pm 0,19$ ) grup 3'ten ( $1,31\pm 0,11$ ) anlamlı olarak yüksekti ( $p<0,05$ ). Ayrıca oksidatif stres indeksi (OSI) grup 3'te anlamlı olarak daha düşüktü ( $p<0,05$ ). Öte yandan nükleer oksidatif stresi gösteren 8-hidroksi-2'-deoksiguanozin (8-OHdG) düzeyleri de grup 3'te düşük bulundu ( $p<0,05$ ).

**SONUÇ:** Sonuçlarımız diyabetik hastalarda kullanılan safen ven greftlerinde endotel solüsyonu kullanımının oksidatif hasarı önlediğini desteklemektedir.

**Anahtar Kelimeler:** diyabetik koroner arter hastalığı, safen greft, oksidatif stres, endotel koruma solüsyonu

## ABSTRACT

**OBJECTIVE:** Saphenous vein grafts used for bypasses of diabetic coronary artery patients are occluded earlier than the normal patient population. In this study, the effects of endothelial protection solution on the protection of saphenous vein grafts taken during the operation against oxidative damage in diabetic patients were investigated.

**MATERIALS AND METHODS:** The remaining parts of the saphenous vein grafts removed during the operation of 50 diabetic patients who underwent coronary bypass operation in our hospital between 2021 and 2022 were divided into three pieces. Each piece was placed into a different solution as saline (group 1), heparinized autologous blood (group 2), and endothelial protection solution (group 3). They were kept in solutions for 4 hours and then cellular and DNA oxidative stress markers were investigated.

**RESULTS:** There was no significant difference between the groups in terms of total antioxidant capacity (TAC) ( $p>0.05$ ). Total oxidative status (TOS) levels in groups 1 and 2 without endothelial solution ( $1.57\pm 0.46$  vs.  $1.55\pm 0.19$ ) were significantly higher than those in-group 3 ( $1.31\pm 0.11$ ) ( $p<0.05$ ). Moreover, the oxidative stress index (OSI) was significantly lower in group 3 ( $p<0.05$ ). On the other hand, 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, which indicate nuclear oxidative stress, were also found to be low in-group 3 ( $p<0.05$ ).

**CONCLUSION:** Our results support that the use of endothelial solution on saphenous vein grafts used in diabetic patients prevents oxidative damage.

**Keywords:** diabetic coronary artery disease, saphenous graft, oxidative stress, endothelial protection solution

## INTRODUCTION

In diabetic patients, uncontrolled and high blood sugar damages the vascular endothelium and heart tissue. Therefore, diabetic patients are not only directly at

increased risk of coronary artery disease (CAD), but are also more likely to have other conditions that cause CAD (1,2).

In addition, when coronary artery bypass graft operation is required in these patients, impaired blood sugar control

also affects graft patency rates after the operation. Although it is stated in the studies that diabetes does not have a direct effect on the long-term patency rates of the internal mammary artery (IMA) graft, there are conflicting studies that indicate the long-term patency negatively affected for the saphenous vein (SV) graft due to uncontrolled diabetes-related blood glucose levels (3-5).

It is already known that the mid-long term patency rates of the saphenous vein are worse than other arterial grafts. For this reason, studies on preventing endothelial damage due to various reasons during graft harvesting, and performing more qualified anastomosis during anastomosis have been designed for obtaining longer patency rates (6). It has been reported that even the high pressure applied during harvesting causes endothelial destruction, and the damage results in acute occlusions by causing platelet adherence and thrombosis in the early period. In the advanced period, it has been observed that endothelial damage that occurs during these events, smooth muscle cell migration is stimulated due to mitogenic proteins released from platelets adhering to the intima, resulting in intimal proliferation and hyperplasia (6,7). Considering that the SV endothelium under this risk is much more sensitive in diabetics, it can be said that these patients are at much higher risk in terms of early and late graft patency rates.

This study, it was aimed to investigate the effects of the solutions in which the saphenous vein is kept during the period until the bypass, on the oxidative stress that the SV endothelium is exposed to in diabetic patients who underwent coronary artery bypass graft (CABG).

## MATERIAL & METHODS

The study was carried out by using the saphenous vein grafts of 50 diabetic patients who applied to Gaziantep University Cardiovascular Surgery Clinic and underwent bypass surgery in 2021 - 2022. The remaining saphenous vein graft after anastomosis was used for biochemical evaluation. Ethical approval was obtained from the local ethics committee before starting this prospective non-randomized controlled study (Approval No. 2021/352). All study steps were planned and implemented in accordance with the Declaration of Helsinki and good clinical practices.

Each residual saphenous vein graft obtained was divided into three parts and placed in three different solutions as follows:

- Saline (0.9 percent NaCl) solution for four hours (Group 1),
- Heparinized Autologous Blood solution for four hours (Group 2),
- Endothelial Protection Solution (NOESIS®, Noegenix, Ankara, Turkey) for four hours (Group 3).

Then, biochemically cellular and DNA oxidative stress levels were studied with commercially available kits.

## Biochemical Analysis

### Total Antioxidant Capacity (TAC)

The antioxidant capacity (TAC) of the endothelial extract was evaluated with commercial kits (Rel Assay Diagnostic, Gaziantep, Turkey) as nmolTroloxEquiv/mg protein as previously described (8,9). In this method, which produces hydroxyl radicals based on the Fenton reaction, the antioxidant capacity of the sample against strong radical hydroxyl is evaluated.

### Total Oxidative Status (TOS)

The oxidative status (TOS) of the extract obtained from the endothelium was evaluated with commercial kits (Rel Assay Diagnostic, Gaziantep, Turkey) as nmol H<sub>2</sub>O<sub>2</sub> Equiv/mg protein as previously described (8,9). The oxidant stimuli in the sample oxidize the iron ion-o-dianisidine complex to the iron ion and the reaction is potentiated with glycerol to evaluate the oxidative stress level.

### Oxidative Stress Index (OSI)

The oxidative stress index (OSI) value over the average of TAC and TOS values is obtained in the Arbitrary Unit (AU) with the following formulation:  $OSI = (TOS, \text{nmol H}_2\text{O}_2 \text{ Equiv/mg protein}) / (TAS, \text{nmol Trolox Equiv/mg protein}) \times 100$  (8,9).

## Nuclear and mitochondrial DNA oxidative damage measurement:

OxiSelect™ Oxidative DNA Damage kit [8-hydroxydeoxyguanosine (8-OHdG) assay, Cell Biolabs, Inc. San Diego, CA] and the data obtained were expressed as ng/ml as described in previous reports (10).

## Statistical Analysis

The SPSS ver. 13.0 was used for statistical analysis of obtained data. Continuous and ordinal data were given as mean and standard deviation, and the normal distribution

was evaluated with the Kolmogorov Smirnov test. Comparison of triplicate data was done with the One Way ANOVA test. For the p values obtained from the comparison, less than 0.05 was considered statistically significant.

## RESULTS

The mean age of the bypassed diabetic patient population was 62.11±10.53, and 30% (n: 15) of the patients were female.

There was no significant difference between the groups (p=0.34) between the TAC values studied from the saphenous vein tissues. However, TOS levels in groups 1 and 2 without endothelial solution (1.57±0.46 vs. 1.55±0.19) were significantly higher than those in group 3 (1.31±0.11) in which endothelial solution was used (p=0.002). OSI was found to be lower in group 3 as a result of the mean between values (p=0.000). Cellular oxidative stress values between groups are summarized in Table 1.

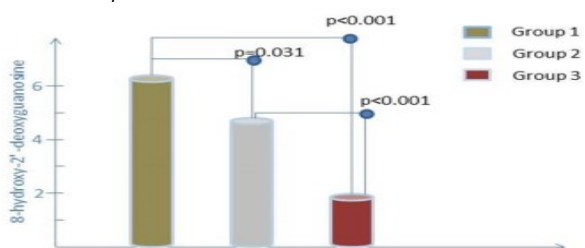
**Table 1.** Cellular oxidative stress markers in groups

Oxidative Markers	Group 1	Group 2	Group 3	P*
TAC** (nmolTroloxEquiv/mg protein)	2.46±0.34	2.41±0.17	2.52±0.31	0.064
TOS*** (nmol H2O2 Equiv/mg protein)	1.57±0.46	1.55±0.19	1.31±0.11	0.002
OSI+ (AU)	0.56 ±0.43	0.52±0.33	0.24±0.07	0.000

\*Groups were compared with One Way Anova test and p<0.05 was accepted as statistically significant, \*\*TAC: Total antioxidant capacity, \*\*\*TOS: Total oxidative status, +OSI: Oxidative stress index

When groups were evaluated in terms of endothelial oxidative DNA damage, the 8-OHdG levels were found as 6.13±1.7 ng/ml in group 1, 4.81±2.40 ng/ml in group 2, and 1.92±0.67 ng/ml in group 3. When the groups were compared, the most severe endothelial oxidative DNA damage was found in group 1 (saline) (Graph 1).

**Graph 1.** The 8-OHdG levels in Groups. Group 1: Saline solution; Group 2: Heparinized blood solution; Group 3: Endothelial protection solution



## DISCUSSION

Our results demonstrated that endothelial protection solution can be beneficial for protecting saphenous vein endothelium against oxidative stress in diabetic patients who underwent coronary bypass. Moreover, our results supported that even a nuclear oxidative stress marker 8-OHdG levels were significantly lower in the endothelial protecting solution applied to harvested saphenous veins.

The processes behind the development of endothelial dysfunction by affecting the endothelial functions in diabetic patients have received increasing attention. Under physiological conditions, the balance between endothelial contraction and relaxation is disrupted in the diabetic patient group, resulting in primary disruption of microcirculation and end-organ damage. Impaired glucose metabolism and associated hyperglycemia have been seen as the main cause of endothelial dysfunction in diabetic patients (11,12). In addition, micro-albuminuria due to end-organ damage, impaired lipid metabolism, and reactive Cu-Zn superoxide dismutase levels caused by acute hyperglycemic attacks are triggering oxidant reactions in diabetes, and creating cumulative oxidative stress on the endothelium that resulting in permanent functional damage (11,12). In Due to endothelial dysfunction, which plays a role in the basic pathogenesis of adverse processes occurring in the diabetic process, it has been emphasized that the protection of the endothelium should be a basic treatment goal (13). Therefore, in this study, the most suitable solution materials that can protect the endothelium by reducing oxidative stress during the period from saphenous harvesting to bypassing were examined.

Ak et al. found intimal fibrosis, endothelial cell vacuolization, and smooth muscle cell damage in the saphenous veins of these patients in their histopathological examination of the LV grafts of diabetic patients who had undergone bypass. However, they reported as a limitation that oxidative stress markers that could cause the pathologies they found in their study were not studied (14). In another report, it was stated that arterial grafts are much more durable than venous grafts in diabetic patients, and it was recommended to use arterial grafts as much as possible for coronary bypass in these patients. However, it has been stated that the use of SV is inevitable in cases where more grafts are required, such as multi-vessel disease, and it is stated that the saphenous vein is more

affected by stress factors that were mentioned in the previous paragraph (15). TAC and TOS measurement and the OSI value obtained by the ratio of these two values are frequently used parameters in the measurement of oxidative stress in tissues and body fluids (16). It has been previously reported that hypoglycemia and hyperglycemia episodes are associated with increased TOS in diabetic patients, and it has been emphasized that this may be due to increased lipid peroxidation measured by TOS/TAC (17). TAC and TOS values were previously evaluated in harvesting SVs and compared in different solutions. That study by Tekin et al. was performed on SVs of patients with or without diabetes, and as a result, it was reported that endothelial protection solution was more protective from oxidative stress than heparinized blood (18). Our study included only diabetic patients and it was determined that the endothelial protection solution protected the vein graft from cellular oxidative stress better than both saline and heparinized blood.

We also investigated the levels of 8-OHdG, a nuclear marker of oxidative stress. 8-OHdG, which occurs metabolically in the oxidative damage of DNA, is a highly sensitive marker for detecting even the smallest nuclear oxidative damage (19). The 8-OHdG levels in urea have also been investigated in diseases caused by occlusive consequences such as vascular dementia and have been accepted as an important indicator of increased oxidative stress. Elevated levels of 8-OHdG in the blood have been associated with an increased risk of atherosclerosis and have been suggested as a predictor of cardiovascular diseases (20,21). In our study, 8-OHdG levels were significantly lower in SVs treated with endothelial protection solution compared to those treated with saline or heparinized blood. This result was interpreted in favor of endothelial protection solution protecting nuclear material against oxidative stress.

In conclusion, our findings supported that endothelial protection solution was more effective than heparinized blood or saline solution in reducing oxidative stress on harvested SV during bypass. In addition, our results suggested that endothelial solution can prevent oxidative stress-induced DNA damage on the SV endothelium.

Limitations of Study: The main limitation of the study is that laboratory analyzes were not confirmed histopathologically. Demonstrating the effects of the obtained data at the cellular level will increase the validity of the results. Another

limitation is that the endothelial solution has only been investigated on the saphenous vein. Demonstrating the same results with other bypass grafts will provide more comprehensive results in terms of endothelial protection efficiency.

Etik: Bu çalışmanın etik kurulu alınmıştır (No. 2021/352).

The ethical approval was obtained from local ethical committee of university (No. 2021/352).

Yazar katkı durumu; Çalışmanın konsepti; EH, ÖA dizaynı; EH, ÖA Literatür taraması; EH, ÖA verilerin toplanması ve işlenmesi; EH, ÖA istatistik; EH, ÖA yazım aşaması; EH, ÖA

Author contribution status; The concept of the study; EH, ÖA design; EH, ÖA literature review; EH, ÖA collecting and processing data; EH, ÖA statistics; EH, ÖA writing phase; EH, ÖA

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