Examination of the internal thoracic artery by transmission electron microscope in coronary artery bypass surgery patients

Koroner arter bypass cerrahisi hastalarında internal torasik arterin transmission elektron mikroskopisi ile incelenmesi

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Background: We presented the histologic findings of clipped and perfused internal thoracic arteries (ITA) examined using a transmission electron microscope (TEM) in patients undergoing coronary artery bypass grafting (CABG) surgery.

Methods: Sixty patients were randomly selected for this study and divided into two different groups according to the ITA harvesting method. In the first group [clipped group (CG); n=38], the ITA was prepared with a pedicle; the distal part was clipped, cut, and covered with a papaverine-soaked cloth until the anastomosis time. In the second group [perfused group (PG); n=22], the graft was not cut after ITA harvesting and was left in the thorax with perfusion until the time of anastomosis. Just before the ITA anastomosis, an ITA ring of 1 mm length was cut and preserved in 2.5% glutaraldehyde solution. The histological descriptions of the samples were done according to the following subgroups: *(i)* completely confluent endothelium; *(ii)* partially confluent endothelium; *(iii)* loosely netted endothelium; *(iv)* islands of endothelium; and *(v)* no endothelium.

Results: In the CG group, different degrees of histopathologic findings were recorded in eight patients (21.05%). In the PG group, endothelial or subadventitial pathology was seen in two patients (9.09%) in the histopathological examinations of ITA. The most important histopathological findings of ITA were as follows: endothelial vacuolization, intimal thickening and/or intimal separation, subendothelial edema, edema in cytoplasma and mitochondria.

Conclusion: Clipping of the ITA after harvesting may damage the integrity of the cell skeleton. Our study showed that the sudden occlusion of the ITA conduits harvested using the standard technique induces a non-physiological condition and may cause an impairment in the endothelial continuity during this pathological process in the endothelium and cellular blood elements.

Key words: Clipping; endothelial damage; histology; internal thoracic artery; tranmission electron microscope.

Amaç: Koroner arter bypass greftleme (KABG) ameliyatı yapılan hastalarda transmission elektron mikroskopisi (TEM) kullanılarak incelemesi yapılan kliplenmiş veya perfüze haldeki internal torasik arter (İTA)'lerin histolojik sonuçları sunuldu.

Çalışma planı: Çalışma için 60 hasta rastgele seçildi ve İTA çıkarılış yöntemine göre iki farklı gruba ayrıldı. İlk grupta [klipleme yapılan grup (KG); n=38] İTA pediküllü olarak hazırlandı, distal bölüm kliplendi, kesildi ve papaverin emdirilmiş spanç içinde anastomoz zamanına dek bekletildi. İkinci grupta [perfüze grup, (PG); n=22] ise İTA çıkarılması sonrasında greft kesilmedi ve anastomoz zamanına kadar göğüs içerisinde perfüse halde bekletildi. İnternal torasik arterlerin anastomozundan hemen önce 1 mm uzunlukta bir ITA halkası kesildi ve %2.5'lik glutaraldehide solüsyonunda muhafaza edildi. Örneklerin histolojik tarifleri belirtilen alt gruplara göre yapıldı: *(i)* Tamamen sağlam endotel; *(ii)* parçalı olarak sağlam endotel; *(iii)* gevşek ağsı yapıda endotel; *(iv)* endotel adacıklı ve *(v)* endotel yokluğu.

Bulgular: Klipleme yapılan grupta sekiz hastada farklı derecelerde histopatolojik bulgu kaydedildi (%21.05). Perfüze grupta İTA histopatolojik incelemelerinde iki hastada (%9.09) endotelyal veya subadventisiyal patoloji saptandı. İnternal torasik arterlerin en önemli histopatolojik bulguları; endotelde vakuolizasyon, intimal kalınlaşma veya intimal ayrılma, subendotelyal ödem, hücre sitoplazması ve mitokondrilerde ödem idi.

Sonuç: İnternal torasik arterin çıkarılması sonrasında kliplenmesi hücre iskeletinin düzeninde bozulmaya yol açabilir. Çalışmamız göstermiştir ki; standart yöntemle çıkarılan İTA konduitlerinde ani yapılan oklüzyon fizyolojik olmayan bir durum oluşturmakta, kan hücre elemanlarıyla endoteldeki bu patolojik süreçte endotel devamlılığında bozulma olabilmektedir.

Anahtar sözcükler: Klipleme; endotel hasarı; histoloji; internal torasik arter; tranmisyon elektron mikroskobu.

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It is now well established that using the internal thoracic artery has a long-lasting impact on survival and function after coronary artery bypass grafting (CABG). Unfortunately, acute graft thrombosis and vasospasm due to intrinsic or extrinsic factors are still severe problems. Endothelial injury during graft harvesting and intimal disease are among these complications. Injury to the internal thoracic artery (ITA) endothelium can activate a coagulation cascade and; therefore, early graft thrombosis and spasm can be seen after surgery.[1] Moreover, the damage to the endothelium can also promote the atherosclerotic process,[2] eventually leading to the long-term development of graft stenosis or occlusion. In traditional techniques, the surgeons prepare the bypass conduit in a pedicle, cutting it at the distal end, occluding it with a clip, and storing it in a vasodilator-soaked tissue until it is implanted. In this study, we used a transmission electron microscope (TEM) to morphologically evaluate the endothelial integrity of clipped and perfused ITA in our CABG patients.

PATIENTS AND METHODS

Preoperatively, informed consent was obtained from all patients. The left ITAs harvested from 60 consecutive patients undergoing elective CABG were the subject of this study. The patients were divided into two groups. In group 1 [clipped group (CG); n=38], the mean age of the patient was 56±4 years (range 38 to 82 years), and 73% of patients were male. A history of smoking was noted in 53% of patients. Most of the patients had multivessel coronary heart disease. Five patients had only left anterior descending artery stenosis. Forty-two percent of cases had hypertension and 56% of cases had diabetes mellitus. Seventy-five percent of patients had been administered oral antidiabetics and the remaining cases had been medicated with insulin. In group 2 [perfused group (PG); n=22], the mean age of the patients was 49±2,3 years (range 27 to 73 years), and 80% of patients were male. A history of smoking was noted in 41% of patients. Most of the patients had multivessel coronary heart disease. Three patients had only left anterior descending artery stenosis. Fifty-three percent of cases had hypertension and 63% of cases had diabetes mellitus. Forty-three percent of patients were administered oral antidiabetics and the remaining cases were medicated with insulin. All patients had been preoperatively treated with acetylsalicylic acid (ASA), beta-blocker agents and nitrates. Patient characteristics, including general data, preoperative hemodynamics and medical managements are defined in table 1.

All ITA conduits were harvested and prepared by an experienced surgeon. After performing a standard midline sternotomy, the left ITA was harvested in a pedicled fashion using low voltage electrocautery. A longitudinal incision on the endothoracic fascia about 1 cm medial to the ITA and its medial satellite vein was made using electrocautery. The fatty tissue around the

Table 1. Composition of clipped group including general data, preoperative hemodynamics and medical treatment

	n	%	Mean±SD	Min.-max.	Range
Number of patients	38				
Age (years)			56±4	38-82	
Height (cm)	158				150-188
Weight (kg)	95				58-1117
Body mass index (kg/m^2)	24				20-35
Mean blood pressure (mm/Hg)			54±7		
Time: preparation to implantation mean	63			31-69	
Flow of internal thoracic artery (ml/min) mean	84			33-220	
Smoker		53			
Hypertension		42			
Diabetes mellitus		56			
Hypercholesterolemia		70			
Beta-blocker therapy		100			
Calcium antagonists		85			
Angiotensin converting enzyme inhibitors		55			
Aggregation inhibitors		100			
Anticoagulants		25			
Coronary vasodilators		100			
Diuretics		20			

SD: Standard deviation; Min.: Minimum; Max.: Maximum.

ITA was not removed in this way. Next, the branches of the ITA were exposed and clearly visualized. At least 2-3 mm away from the ITA itself, the branches were clipped; thus, we were able to divide the branch. During conduit harvesting, care should be taken that the elecrocautery does not come in contact with the ITA. In this way, the ITA is fully harvested from its origin to 1 cm beyond the bifurcation. In the CG, after administration of a full dose heparin, the terminal portion of the ITA just proximal to the bifurcation was cut and clipped. In order to provide ITA dilatation, we did not use any instrument or manipulation. Papaverine solution was applied into the endothoracic fascia using a number 20 thin needle. The ITA pedicle was wrapped in a soaked gauze pad and banked under the left sternal space until anastomosis to the left anterior descending (LAD) artery. Cardiopulmonary bypass was instituted. After the completion of venous graft anastomosis, the mean arterial pressure was noted, and 1-2 mm length of ITA was cut for TEM study to evaluate its endothelial integrity and for an electron microscopic examination.

In the PG, the arteries were prepared in a pedicle and remained connected to the blood flow in situ until implantation. Papaverine was also used in this group. A topical vasodilator was injected into the periarterial tissue using a number 20 thin needle. After systemic heparin administration, cardiopulmonary bypass was instituted. Venous graft anastomoses were completed. We did not apply any retraction or compression using forceps. The mean arterial pressure was noted, and the ITA was cut just proximal to the bifurcation and a 1-2 mm ITA ring was obtained as a TEM sample before applying the bulldog clamp. Afterwards, the bulldog clamp was applied and ITA to LAD anastomosis was performed.

The time between the end of ITA preparation and anastomosis to the LAD artery was determined in each group.

Preparation for transmission electron microscopy

The ITA cylinders were cut longitudinally, immediately washed gently with a physiologic solution, and immersed in 2.5% glutaraldehyde for 24 hours. All samples were fixed in 2.5% glutaraldehyde for 24 hours, washed in a phosphate buffer (PH=7.4), and postfixed in 1% osmium tetroxide (OsO_4) in a phosphate buffer. Thereafter, it was, further dehydrated in ascending concentrations of ethyl alcohol. Then the tissues were washed with propylene oxide and placed in epoxy-resin embedding media. Semi-thin sections about 2 μm in thickness and ultrathin sections about 60 nm in thickness were cut with a glass knife on a LKB-Nova (Ultrotome NOVA, LKB, Bromma, Sweden) ultramicrotome. Semi-thin sections were stained with methylene blue and examined by a Nikon Optiphot (Nikon, Optiphot, Tokyo, Japan) light microscope. Ultrathin sections were collected on copper grids, stained with uranyl acetate and lead citrate, and examined with a Jeol JEM 1200EX (JEOL Ltd., Tokyo, Japan) transmission electron microscope. One blinded anatomo-pathologist examined all specimens and described the endothelial integrity using the following criteria (3): *(i)* completely confluent endothelium; *(ii)* partially confluent endothelium; *(iii)* loosely netted endothelium; *(iv)* islands of endothelium; and *(v)* no endothelium.

Statistical analysis

Statistical analysis was carried out by the use of SPSS for Windows Version 11.0 software (SPSS Inc., Chicago). Variables were presented as mean ± standard deviation. The normal distribution of variances among groups for continuous variables was evaluated by Levene's test. The chi-square test was used to compare dichotomous variables, and the Student t-test was used for continuous variables. *P* values ≤0.05 were considered statistically significant.

RESULTS

The patient groups were compared for mean age, body weight, and time until anastomosis; no significant differences were found (Table 1). Risk factors and preoperative medication use were not significantly different between the groups. Just before the ITA anastomosis, the anaesthesiologist recorded the mean arterial blood pressure. The mean blood pressure in the perfused and clipped group was 54±7 mmHg, and 51±6 mmHg, respectively. When we compared the mean blood pressure, there was no statistical significance between the groups ($p>0.05$). The mean time of the preparation until LAD anastomosis was 63 min., and 54 min., in PG and CG, respectively. There was no statistical difference between the two groups ($p>0.05$). In the CG of patients, ITA mean blood flow was 84 mil./min. In the PG, mean ITA flow was 75 mil/min. No statistical difference was found between the groups ($p>0.05$).

In the CG, in eight cases (21.05%), there were different degrees of pathological conditions in the ITA examination. In the remaining thirty CABG patients, there was no evidence of ITA pathology (Figure 1). In the PG, we did find different degrees of ITA endothelial pathology in two cases (9.09%). These two cases were older than 70 years and also had insulin-dependent diabetes mellitus. When we compared the ITA pathology, there was a statistical difference between the two groups ($p<0.05$). Our study showed that vascular

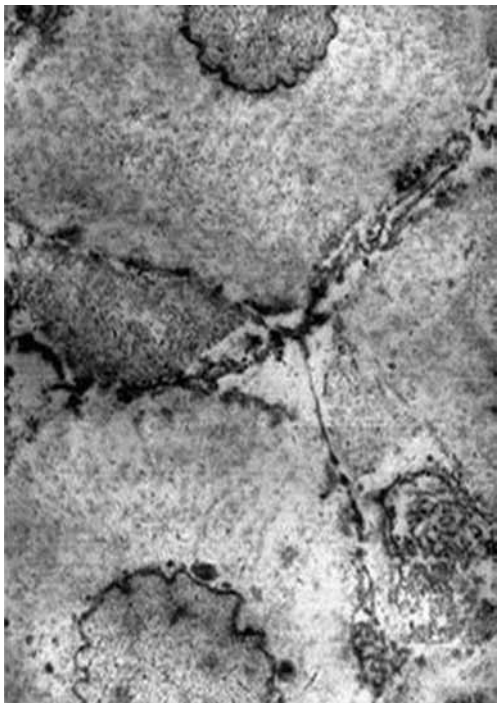

Figure 1. This demonstrates the normal endothelial composition of the ITA conduit. There is no gap or seperation between the cells and no cellular matrix corruption is seen (Metilen blue 1 x 12000).

pathology was more frequent than the PG. Statistical results and *p* values are summarized in Table 2. Patients characteristic are summarized in table 3.

One of the main pathological findings in these tissues was a significant decrease in smooth muscle cells of tunica media observed in the volume of intracellular organelles. We also detected swollen mitochondria in the endothelium (Figure 2). There was a heterochromatine rich nucleus in these cells. The cytoplasm of these cells was swollen due to the collection of fluid. Subendothelial vacuolisation and endothelial cell separation was seen in three of eight patients in the clipped group (Figure 3). We also saw that there was an island cell due to severe endothelial separation and a heterochromatin-rich nucleus in the same three patients in the clipped group. Edematous areas were present around the external elastic membrane in tunica media and tunica adventitia (Figure 4). Loss of cells and basal lamina were present around the internal elastic membrane (Figure 5). Cellular damage and separation was found between the smooth muscle cells of tunica media and tunica adventitia (Figure 6).

We saw that in many of the samples, cellular edema was the main pathological finding. However, in the clipped group, a decrease of cellular organelle volume in the smooth muscle cell area was found.

DISCUSSION

It is now well established that using the ITA has a long-lasting impact on survival and function after coronary artery bypass grafting.[3-5] However,

Table 2. Composition of perfused group including general data, preoperative hemodynamics and medical treatment

	n	%	Mean±SD	Min.-max.	Range
Number of patients	22				
Age (years)			49±2.3	27-73	
Height (cm)	146				152-176
Weight (kg)	81				62-112
Body mass index (kg/m^2)	22				19-31
Mean blood pressure (mm/Hg)			51±6		
Time: preparation to implantation mean	54			27-58	
Flow of internal thoracic artery (ml/min) mean	75			25-133	
Smoker		41			
Hypertension		53			
Diabetes mellitus		63			
Hypercholesterolemia		53			
Beta-blocker therapy		100			
Calcium antagonists		90			
Angiotensin converting enzyme inhibitors		65			
Aggregation inhibitors		100			
Anticoagulants		5			
Coronary vasodilators		100			
Diuretics		15			

SD: Standard deviation; Min.: Minimum; Max.: Maximum.

Table 3. Statistical analysis of patients characteristics

	Clipped arteries			Perfused arteries			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Number of patients	38			22			
Number of histologyc pathology	8			2			0.0235
Mean age, (years)			56±4			49±2.3	0.143
Mean height, (cm)	158			146			0.980
Weight, (kg)	95			81			0.540
Time until implantation	63			54			0.171
Percent of smokers		53			41		0.680
Percent of patients with hypertension		42			53		1.0
Percent of IDDM		63			56		0.965
Percent of hypercholesterolaemia		53			70		0.670
Percent of patients taking beta-blocker		100			100		1.0
ACE inhibitor		55			65		0.85
Ca-channel blocker		85			90		1.0
Antiaggregant therapy		100			100		1.0

SD: Standard deviation; IDDM: Insuline dependent diabetes mellitus; ACE: Angiotensin converting enzyme.

thrombosis or vasospasm of the ITA in the early postoperative period[6,7] and development of intimal proliferation and atherosclerosis years after CABG surgery have previously been reported.[6] Both of these problems may lead to adverse outcomes for the patient. In a previous study that used scanning electron microscopy, endothelial cell derangements indicated endothelial cell dysfunction in ITA grafts that were harvested by the traditional clipped artery technique.[8]

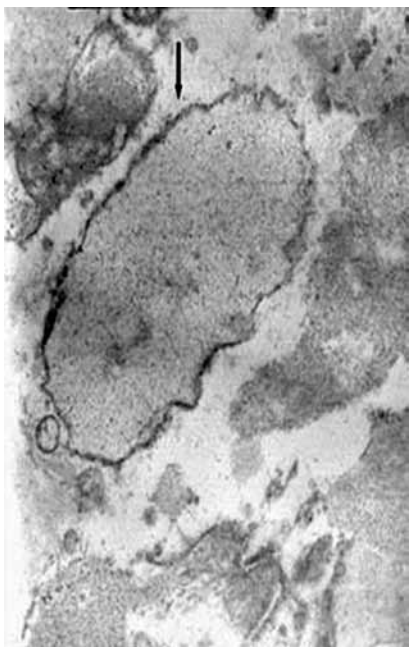

Figure 2. The black arrow shows the mitochondria from the endothelial cell. This image was selected from the severely damaged and seperated vascular layer due to severely disorganised cytosceletal structure from the clipped group (Metilen blue 1 x 15000).

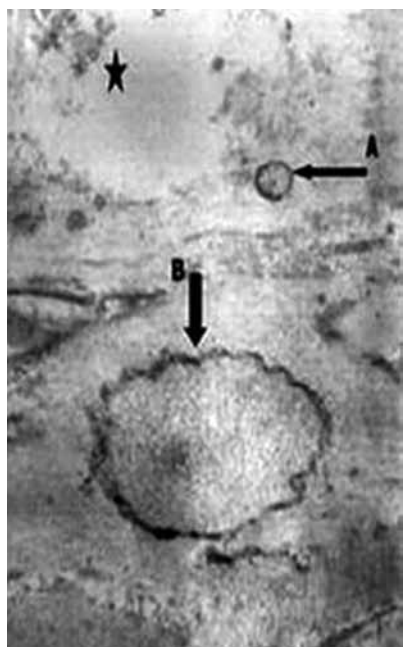

Figure 3. Arrow A is exhibiting small vacuolisation in the endothelial cell (from the clipped group). There is a heterochromatine rich nucleus in these cells (arrow B). The black star shows collection of fluid in the cytoplasm of these cells (Metilen blue 1 x 15000).

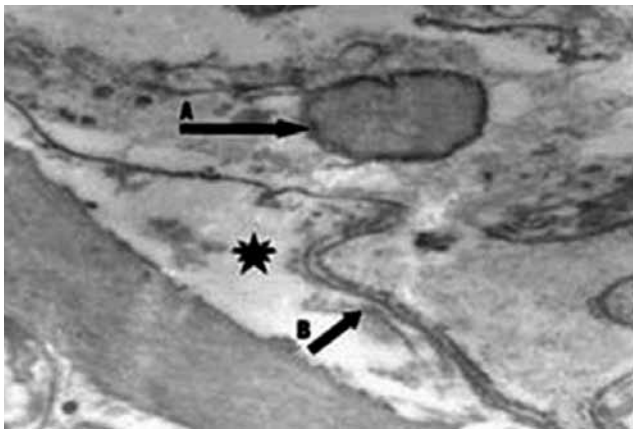

Figure 4. This shows the endothelial seperation and heterochromatin rich nucleus (arrow A). A separated area is indicated by a black star, and arrow B shows the separated cell wall (Metilen blue 1 x 10000).

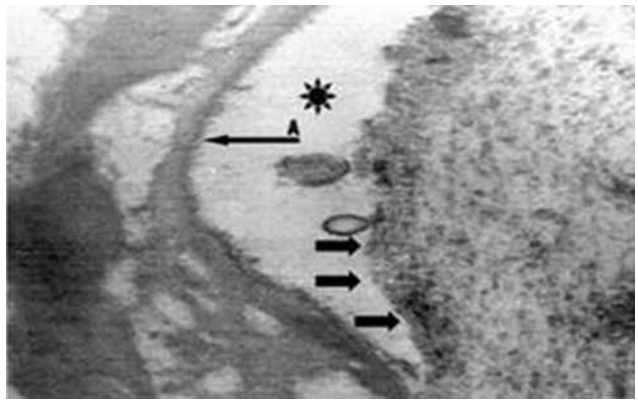

Figure 5. This shows the loss of basal lamina which is present around the internal elastic membrane (Arrow A). This figure also shows intimal thickening, intimal separation and derangements (small arrows). The black asterisk symbol shows the separated internal elastic membrane (Metilen blue 1 x 15000).

As we know, ITA conduit failure depends largely on the harvesting method. A number of surgeons prefer clipping the ITA and banking it under the left sternal space for a long time (from the harvesting to anastomosis). This approach causes intraluminal high pressure and endothelial damage along with thrombogenic cascade activation.[9]

In the present study, to investigate the side effects of clipping the ITA, we morphologically assessed the endothelial integrity and cytoskeletal ultrastructure using TEM. In our study, skeletonization of the ITA was not performed and all conduits were harvested in the pedicled fashion. No injury to the ITA, such as dissection or weak bleeding from the conduit, was detected. All grafts were pulsatile, and all ITA blood flow was good. In our CG, there were different degrees of ITA wall pathology in eight patients. In the CG, three of eight patients had diabetes mellitus, and five of eight cases were more than 70 years old. In the second group, only two patients had insulin-dependent diabetes mellitus. They were also more than 70 years old. Intercellular derangements and separation of the vascular wall were the more frequent clipped ITA pathologies in our study. Although there was cellular damage in two cases in the PG, we did see that the ITA wall pathology was more severe in the CG. Intercellular separation, organelle edema and severely disorganised vascular walls were high in the CG. Depositions of cellular remnants and fibers were more frequently seen in our CG.

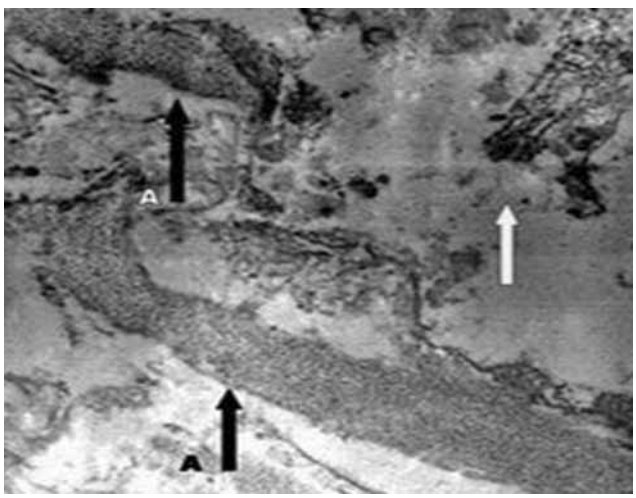

Figure 6. This shows edematous areas around the external elastic membrane in tunica media and tunica adventitita. The white arrow shows the edematous area (from the clipped group). In addition, this figure shows the loss of basal lamina around the internal elastic membrane (white A arrow). The black A arrow is demonstrating disorganised basal membrane (Metilen blue 1 x 15000).

In the previous investigation, it was shown that cellular loss and damage of intercellular regions could be seen in the clipped ITA.[10] In this study, Grapow et al.[10] found that the concentrations of sP-selectin and thrombomodulin were significantly higher in plasma from clipped arteries compared to perfused arteries. Scanning electron microscopic examination revealed significant structural changes and loss of endothelial cells in clipped arteries in three of their patients. In contrast to perfused ITA, our work showed that the clipping of the arterial conduit may cause severe endothelial cell damage and cell loss with exposure of the basal lamina. We know that the arterial endothelium plays an important role in vascular tone since it releases autocrine and paracrine substances, such as internal vasodilators and prostacycline. To mediate vasodilation, the endothelium exerts antiatherogenic effects through

the potent inhibition of platelet aggregation, smooth-muscle proliferation, and leukocyte adhesion.[10,11]

In the traditional technique, the surgeon prepares the bypass conduit in a pedicle by cutting it at the distal end, occluding it with a clip, and storing it in a vasodilator-soaked tissue until it is implanted. This procedure, in general, necessitates longer lengths of time, especially in multivessel CABG operations. Thus, the pressure of the ITA lumen is increased, and some mediators, such as selectin family members, are excreted and nitric oxide levels are decreased. In our investigation, the time period between the harvesting and anastomosis of the ITA conduit was 63 (range 31 to 69) minutes. The time period from harvesting to anastomosis was short in cases with single coronary artery disease (five cases). However, in three of five patients, ITA pathology was recorded. In our opinion, endothelial damage might depend on increased intraluminal mediators (selectin family) and the amount of time between the harvesting and anastomosis of the ITA circuit. The duration of ischemia may play a main role in the triggering of the arterial conduit pathology.

The other problem is the use of harvesting devices during the ITA preperation. A histological study confirmed that different devices such as an ultrasonic scalpel or high frequency electrocautery application on the branches from 1 to 5 mm away from the ITA itself did not cause any endothelial injury.[12-15] Grapow et al.[10] have previously compared the two groups of ITA using different harvesting techniques, including clipped and perfused ITA storage In this study, the authors showed that leaving the ITA perfused during harvesting could considerably improve the endothelial function of the conduit. They have reported that the clipped ITA had higher contractile responses compared to perfused ITA. However, they did not report the endothelial integrity and ultrastructural changes of these conduits.[8] Thus, the author believed that we need large, multicentric studies to see the outcome of relationships between vascular pathology and harvesting techniques.

In conclusion, the vascular endothelium is of primary importance in maintaining the viability and patency of the ITA. In the present study, we morphologically and ultrastructurally assessed the endothelial integrity of the clipped ITA using TEM in our CABG cases. There is no report about the ultrastructural changes, including intracellular organelles and cellular pathology in clipped ITA using a TEM in a large case series. Surgical manipulation, storage conditions, and distension before anastomosis can abnormally alter the antithrombogenic property of the endothelium, leading to vasospasm, thrombogenesis, occlusive intimal hyperplasia, and stenosis. Intimal hyperplasia occurs as a consequence of physiological stimuli, constituting an attempt by the tissue to maintain normal conditions of flow, wall tension, or both. Our study findings show that ultrastructural conduit pathology, including subadventitial region, cellular and intercellular relation, is damaged by the clipping of the ITA.

Declaration of conflicting interests

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