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Research Article

Impact of age on ultrastructural changes in internal thoracic artery in patients undergoing coronary artery bypass grafting

Koroner arter baypas greftleme yapılan hastalarda yaşın internal torasik arterdeki ultrastrüktürel değişiklikler üzerine etkisi

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Abstract

Aim: Age is a known risk factor for cardiovascular disease and plays a role in the atherosclerotic process. The purpose of this study was to look at the impact of aging and associated risk factors on the ultrastructure of internal thoracic arteries in patients undergoing coronary artery bypass grafting.

Material and Methods: We enrolled 27 patients undergoing elective coronary artery bypass grafting at our institute between August-October 2018. Age groups were classified into three: 50-59, 60-69, and 70-79 years. A 2-mm distal portion of the vessel was excised when the ITA was surgically harvested. Transmission electron microscopy was used to investigate the ultrastructural changes. Cell structure, tissue edema, and endothelial mitochondria were all assessed and rated by using semiquantitative analysis.

Results: The ultrastructure of the vessel wall exhibited no significant changes in Group-I. Endothelial wall irregularity with endothelial cells of varying thickness was seen in Group-II. Group-III showed subendothelial edema and localized endothelial wall discontinuity. These changes were particularly severe in the elderly and patients with comorbidities. The greatest permanent cell alterations, such as massive vacuoles and organelle loss, were identified in two patients with kidney failure and hypertension. The average scores assessing the severity of changes in endotohelial cell structure ($P < .001$), tissue edema ($P < .001$), and mitochondria ($P < .001$) were significantly different between groups showing more severe changes with aging.

Conclusions: In elderly patients with comorbidities, the ITA endothelium may exhibit severe ultrastructural alterations, with the most permanent abnormalities reported in those with hypertension and kidney failure. However, The ITA remains the gold standard in CABG with its native resistance to atherosclerosis.

Keywords: Internal thoracic artery, transmission electron microscopy, coronary artery disease, aging

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Öz

Amaç: Yaş, kardiyovasküler hastalık için bilinen bir risk faktörüdür ve aterosklerotik süreçte rol oynar. Bu çalışmanın amacı, koroner arter baypas greftlemesi yapılan hastalarda yaşlanmanın ve ilişkili risk faktörlerinin internal torasik arterlerin altyapısı üzerindeki etkisini incelemektir.

Gereç ve Yöntemler: Ağustos-Ekim 2018 tarihleri arasında birimimizde elektif koroner arter bypass ameliyatı geçiren 27 hasta çalışmaya dahil edildi. Yaş aralıkları 50-59, 60-69 ve 70-79 olmak üzere üç gruba ayrıldı. ITA cerrahi olarak çıkarıldığında damarın 2 mm'lik distal kısmı eksize edildi. Ultrastrüktürel değişiklikleri araştırmak için transmisyon elektron mikroskobu kullanıldı. Hücre yapısı, doku ödemi ve endotelyal mitokondri yarı kantitatif analiz kullanılarak değerlendirildi ve derecelendirildi.

Bulgular: Grup-I'de damar duvarında ultrastrüktürel anlamlı bir değişiklik görülmedi. Grup-II'de değişen kalınlıklarda endotel hücreleri içeren endotel duvar düzensizliği görüldü. Grup-III'de subendotelyal ödem ve lokalize endotel duvar süreksizliği görüldü. Bu değişiklikler özellikle yaşlılarda ve eşlik eden hastalıkları olan hastalarda şiddetliydi. Büyük vakuoller ve organel kaybı gibi en büyük kalıcı hücre değişiklikleri, böbrek yetmezliği ve hipertansiyonu olan iki hastada tespit edildi. Endotelyal hücre yapısındaki (P = 0,001), doku ödemi (P = 0,001) ve mitokondrideki (P = 0,001) değişikliklerin ciddiyetini değerlendiren ortalama puanlar, yaşlanmayla birlikte daha ciddi değişiklikler gösteren gruplar arasında anlamlı derecede farklıydı.

Sonuç: Eşlik eden hastalıkları olan yaşlı hastalarda, ITA endoteli ciddi yapısal değişiklikler sergileyebilir; en kalıcı anormallikler hipertansiyon ve böbrek yetmezliği olanlarda rapor edilmiştir. Ancak ITA, ateroskleroza karşı doğal direnci nedeniyle KABG'de altın standart olmaya devam etmektedir.

Anahtar Kelimeler: İnternal torasik arter, transmisyon electron mikroskobu, koroner arter hastalığı, yaşlanma

Introduction

Due to the aging of the global population, cardiovascular diseases have emerged as prominent causes of mortality on an international scale. Major risk factors include advanced age, diabetes mellitus, hypertension, hyperlipidemia, smoking, and kidney disease [1,2]. Along with these risk factors, clinical implications of inflammation, arterial rigidity, and vascular endothelial dysfunction are significant [3-5].

As numerous observational studies have shown, the left internal thoracic artery (ITA) grafted to the left anterior descending artery (LAD) provides an optimal, reliable conduit. The ITA has demonstrated resistance to atherosclerosis and, when utilized for grafting, decreases the occurrence of significant adverse cardiac events, improves event-free survival, and yields satisfactory graft patency outcomes in comparison to the radial artery or saphenous vein in coronary artery bypass grafting (CABG) [4-6]. Nevertheless, certain histologic studies have identified both early and advanced atherosclerotic lesions, with the intima being the most significantly affected, in ITA grafts from patients with multiple risk factors [7]. Consistent with the steady increase in the life expectancy, our clinical practice increasingly involves frail elderly patients who present with multiple comorbidities and risk factors for CABG [8, 9].

The purpose of this investigation was to determine, through ultrastructural analysis, the impact of aging and associated risk factors on ITA grafts in patients who had undergone CABG.

Material and Methods

Study design and data collection

Twenty-seven patients who underwent elective CABG at our institute between August and October 2018 were included in this study. To facilitate comparison of ultrastructural findings and effective interpretation of the results, the patients were separated into three age groups. Group I included nine patients aged 50-59, Group II included ten patients aged 60-69, and Group III included eight patients aged 70-79 years. Exclusion criteria were; patients with left ventricular ejection fraction less than 40%, age younger than 50 years, ager older than 79 years, patients who underwent emergency CABG, patients who had previously undergone cardiac surgery, patients with severe peripheral vascular disease, and patients which did not receive ITA graft.

Sample collection

ITA harvesting was standardized and protocolized during the study period. After midline sternotomy, the left ITA was harvested as a pedicle using a 20 W monopolar electrosurgical electrode (KLS Martin, Gebrüder Martin GmbH & Co. KG, Tuttlingen, Germany) from its origin at the subclavian artery to its distal bifurcation with the large intercostal branches clipped. Following the administration of systemic unfractionated heparin (300 U/kg), ITA pedicle was treated with topical papaverine to prevent vasospasm, and the graft was seperated from its distal bifurcation site. Tissue samples of approximately two mm were collected from the distal end of the graft. It is known that ITA segments distal to bifurcation are more prone to intimal hyperplasia and atherosclerotic changes. However, we think it is not clinically relevant as these segments are not used in CABG surgery.

Tissue preparation, ultrastructural and semiquantitative analysis

For electron microscopy studies, ITA tissues obtained at the time of CABG was immediately minced into one to two mm pieces and fixed in cold-buffered 2.5% glutaraldehyde solution for 24 hours. A post-fixation procedure was performed in 1% osmium tetroxide solution after the samples were irrigated with Sorenson's Phosphate Buffer (SPB) solution. After the fixation process, samples were irrigated with SPB for the second time and dehydrated with increasing concentrations of alcohol. Samples were then irrigated with propylene oxide and were embedded in the epoxy resin embedding media, and they were left in the incubator for 48 hours. Ultrathin sections, 60nm, were cut with a glass knife on an LKB-Nova Ultramicrotome (Nova, Bromma, Sweden). These ultrathin sections were collected on copper grids, stained with uranyl acetate and lead citrate. The ultrastructural analysis was made with an electron microscope (JEOL JEM EX, Tokyo, Japan) and photographs were obtained. Apart from the morphologic analysis of the tissue samples, slides of each ITA were analyzed with a semiquantitative method by a histopathologist blinded to the study (magnifications 10000×). This scoring method is an original method developed by our histopathologist and several papers have been published by using this methodology [3, 9, 11]. In each artery sample, ten different areas on the slides were evaluated and scored. Endothelial cell structure was scored as follows: 0, normal thickness; 1, decreased thickness of endothelial cells; 2, discontinuities in the endothelial wall; 3, complete damage of endothelial wall. Degree of tissue edema was scored as follows: 0, no edema; 1, edema in the endothelial layer; 2, edema in the endothelium and subendothelium; 3, edema in the endothelium, subendothelium, tunica media, and tunica adventitia. Ultrastructural changes in endothelial mitochondria were scored as follows: 0, normal (ultrastructurally normal mitochondrion); 1, mild (mild degree of swelling, mitochondrion with prominent cristae); 2, moderate (swollen mitochondrion, cloudy swelling); 3, severe (amorphous material deposition in the mitochondrion).

Definitions of Clinical Variables

A resting systolic blood pressure of ≥140 mmHg and a diastolic blood pressure of ≥90 mmHg were considered systemic arterial hypertension, as with a history of high blood pressure or the requirement for antihypertensive treatment. Diabetes mellitus was described as having a diabetes medical history and being currently treated with insulin or oral medicines. A history of dyslipidemia diagnosed and/or treated by a physician was defined as dyslipidemia. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Kidney Disease algorithm to assess kidney function. The greatest serum creatinine level obtained within two days before surgery was used as the preoperative creatinine level [12]. KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease (CKD) was used to diagnose and grade the severity of CKD.

Statistical Analysis

Statistical analysis of the data obtained in the study was performed using SPSS statistical software version 26.0 for Windows. Continuous variables are expressed as mean ± SD. A one-way analysis of variance was used to compare the numerical data conforming to a normal distribution after the conformity of numerical data to a normal distribution was evaluated using the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare the numerical data that did not fit a normal distribution. The Pearson chi-square test or Fisher exact test was used to compare categorical data. Statistical significance was set at $P < .05$.

Results

Patient Characteristics

Table 1 shows the demographic data and risk factors of the patients. The mean age of the patients in Group I, II and III were 54.33 ±2.7 years, 63.8 ± 3.5 years, and 72.39 ± 3.1 years, respectively. The mean glycated hemoglobin (HbA1c) level was 8.13% ± 0.7% in Group I, 7.87% ± 0.7% in Group II, and 8.05% ± 0.8% in Group III. The gender distribution, number of patients with hypertension, hyperlipidemia, and history of smoking were not statistically different between groups. There was one male CKD patient in Group II and another

female patient in Group III. eGFR levels and KDIGO CKD grades were 18 (Grade 4) and 14 (Grade 5), respectively.

Table 1. Demographics of patients enrolled in the study.

	Group I (n=9)	Group II (n=10)	Group III (n=8)	p
Age	54.33 ± 2.7	63.8 ± 3.5	72.39 ± 3.1	<0.001
Gender (M/F)	8/1	7/3	4/4	0.253
DM	5	4	2	0.663
HbA1c	8.13 ± 0.7	7.87± 0.7	8.05± 0.8	0.960
Hypertension	2	7	4	0.238
Hyperlipidemia	5	5	1	0.183
Smoking	4	3	2	0.468
CKD	0	1	1	0.723

CKD: Chronic kidney disease; DM: Diabetes Mellitus; F: Female; HbA1c: Glycated hemoglobin; M: male.

The ultrastructural findings

The ultrastructure of the vessel wall was normal in Group I (Figure 1). However, the predominant ultrastructural findings in Group II were irregular endothelium walls with endothelial cells of varying thickness showing heterogeneous pattern (Figure 2). In addition to the alterations seen in Group II, subendothelial edema in focal areas and endothelial wall discontinuity were detected in in Group III (older age group). Diabetes mellitus, hypertension, hyperlipidemia, and renal failure were all evaluated in relation to the ultrastructure of the arterial wall. It was noteworthy to see that severe endothelial and subendothelial ultrastructural alterations were detected in two group III patients with diabetes mellitus and in another patient with DM and HT in group III (Figure 3A). Furthermore, in two patients with CKD from group II and III, irreversible cell deterioration with large vacuoles and reduced organelles in endothelial cells were identified and rated as the most significant permanent abnormalities (Figure 3B).

Semiquantitative Electron Microscopic Scores

Ultrastructural examination of ten different locations on the slides for each artery was undertaken, with scores ranging from 0 to 3. In Group III (older age group, semiquantitative analysis revealed endothelial wall disruption, large vacuoles, reduced organelles, enlarged mitochondria in endothelial cells, and mainly diffuse edema. Group III patients had the highest scores in endothelial cell structure, tissue edema and endothelial mitochondria alterations. Patients in Group II had also higher scores than those in Group I. Average scores assessing the severity of changes in endothelial cell structure (P<.001), tissue edema (P<.001), and endothelial mitochondria (P<.001) were significantly different between groups showing more severe ultrastructural changes with aging (Table 2).

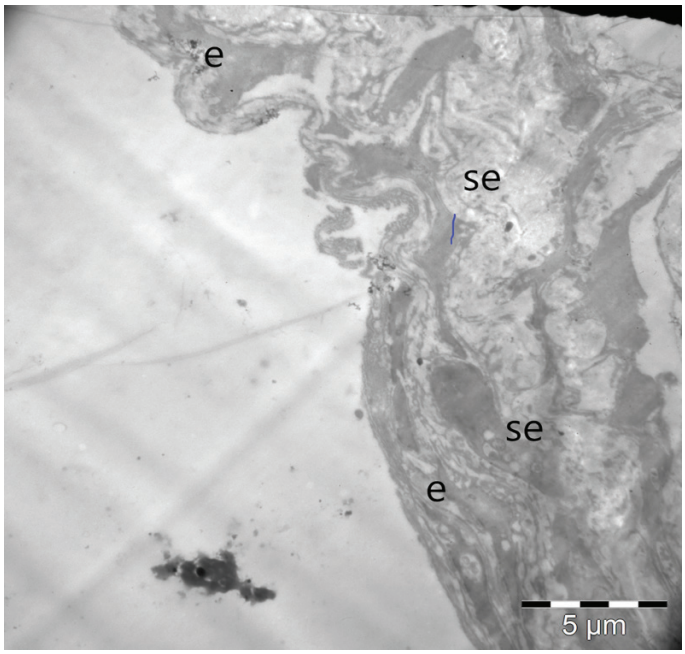

Figure 1. Normal ultrastructural wall appearance in electron microscopy. Electron micrograph of a 57-year-old male patient with no risk factors showing ultrastructurally normal endothelium (e) and subendothelium (se). Original magnification, ×5000.

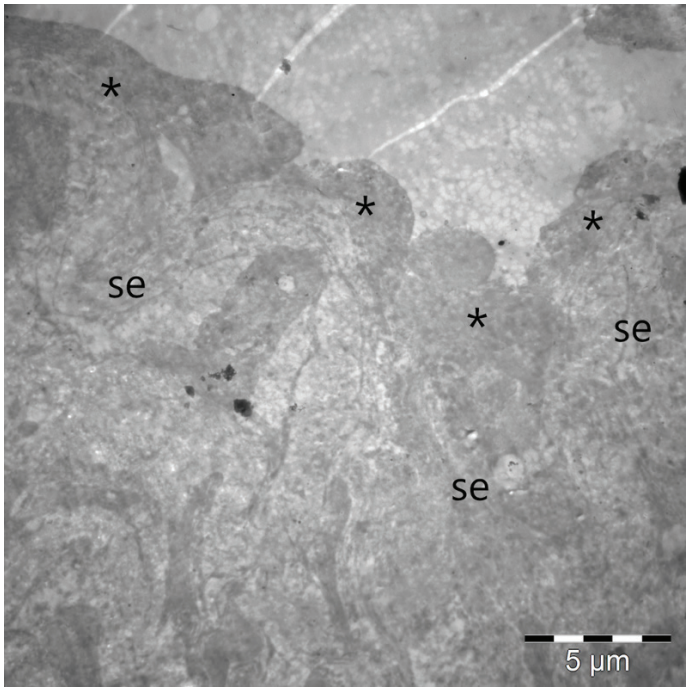

Figure 2. Irregular ultrastructural wall appearance in electron microscopy. Electron micrograph of a 68-year-old female patient with diabetes mellitus and hypertension showing irregular endothelial wall with heterogeneous pattern (*) and ultrastructurally normal subendothelium (se). Original magnification, ×5000.

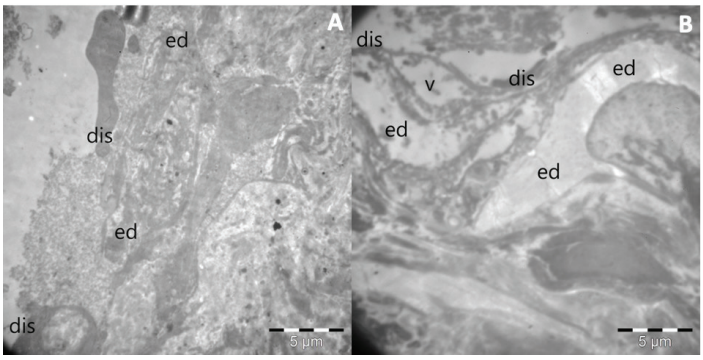

Figure 3. Severe ultrastructural wall alterations in electron microscopy. **(A)** Electron micrograph of a 73-year-old male patient with diabetes mellitus showing subendothelial edema (ed) and discontinuation of endothelial wall (dis). Original magnification, ×5000. **(B)** Electron micrograph of a 72-year-old male patient with chronic kidney disease, hypertension and hyperlipidemia showing subendothelial edema (ed), large vacuoles, and loss of organelles in endothelial cells (v) and discontinuation of endothelial wall (dis). Original magnification, ×5000.

Table 2. Semiquantitative ultrastructural scores.

	Group I (n=9)	Group II (n=10)	Group III (n=8)	p
Endothelial Cell Structure	0.33 ± 0.5	1 ± 0.7	2.13 ± 0.6	<0.001
Degree of Tissue Edema	0.67 ± 0.5	2.3 ± 0.48	2.8 ± 0.46	<0.001
Endothelial Mitochondria	1.78 ± 0.44	2.5 ± 0.53	2.75 ± 0.46	<0.001

Discussion

ITA's histologic and morphologic characteristics make it resistant to atherosclerosis. In the previous three to four decades, ITA grafting has been the gold standard revascularization technique for severe LAD stenoses [8, 13]. When angiography was conducted in symptomatic patients who had previously undergone CABG surgery, observations of blocked ITA graft with patent saphenous vein grafts were quite uncommon in standard surgical practice. Harskamp et al. (2016) discovered an 8.6% ITA failure rate in patients who underwent ITA-LAD revascularization and had angiographic follow-up at 12 to 18 months, with low-grade LAD stenosis, competitive flow, and not having diabetes mellitus being predictors of ITA graft failure [14]. It is also well established that aside from ITA morphology, target vascular region, diameter, and degree of stenosis were critical criteria in determining long-term graft patency in CABG. Despite few research has been published on the ultrastructural abnormalities seen in ITAs, particularly in relation to the clinical profile of the patients, the evidence remained limited.

The predominant ultrastructural findings on transmission electron microscopy examination of the left ITA in this investigation were endothelial wall denudation, endothelial cell irregularity, focal loss of endothelial wall continuity, and subendothelial edema. Large vacuoles and a decreased number of organelles were also identified as permanent alterations in two patients with kidney failure and hypertension. These ultrastructural findings are relevant for patients undergoing CABG surgery as these ultrastructural alterations emphasize the importance of postoperative medical treatment to regulate atherosclerotic process through tight control of modifiable risk factors.

We employed the examination of endothelial integrity, tissue edema, and endothelial mitochondria using a semiquantitative scoring methodology of the specimens, which was similar to prior morphological studies using the same method in the literature [3, 10, 11]. The greatest scores were obtained by two patients who had kidney failure and hypertension. In their histomorphological investigation, Kinoshita et al. (2013) examined ITA specimens from 72 CABG patients and discovered that older age and lower eGFR were independently linked with intimal hyperplasia (odds ratio of 1.90 per 10-year increase) [15]. Ruengsakulrach et al. (1999) evaluated ITA samples from 110 CABG patients and reported that intimal hyperplasia was found in nearly 69% of ITAs, atherosclerotic lesions were found in only 0.7% of ITAs, and plaque formation or medial calcification was not detected [16]. Similarly, in this study, we found a decrease in the thickness of the lining endothelial cells as well as a loss of endothelial wall integrity. However, we did not demonstrate any atherosclerotic changes in the analyzed ITA specimens.

In-vitro techniques have traditionally been employed for histomorphological evaluation of vascular grafts in CABG and cadavers by histology, histochemistry, immunohistochemistry, immunofluorescence, and ultrastructural studies. Borovic et al. (2013) proposed that the ITA is a transitional artery since it lacks a consistent structure and has varying medial content along its length. In their morphometric research, they demonstrated that the slow degeneration of the elastic skeleton developed predominantly with aging, but not with atherosclerosis [17]. Numerous investigations have found that both aging and atherosclerotic diseases reduce the ITA's elastic lamellae thickness while increasing fenestrations. In our study, the severity of the changes increased with age, with the most severe abnormalities found in older patients with hypertension and diabetes. Patients with kidney failure and hypertension have had the most severe and irreversible effects.

Arciniegas et al. (1998) were the first to describe the ultrastructure of atherosclerotic plaques on the ITA [18]. Later, Perrotta et al. (2014) found significant alterations in the walls of two patients' ITA specimens, primarily impacting the intima and media [19.] Furthermore, Bakuy et al. (1998) shown that uncontrolled diabetes mellitus with high HbA1c levels is a strong predictor of ultrastructural histopathological abnormalities such as subendothelial edema and mitochondrial modifications [3]. Ultrastructural alterations and increases in intima-media thickness in ITA samples from CABG patients were highly linked with classic cardiovascular risk factors such age, hypertension, diabetes, and other atypical risk factors like kidney failure [7]. Tyrrell and Goldstein (2020) define the pathophysiology of aging as a complex system including extrinsic and intrinsic vascular variables in their review study. IL-6 signaling and inflammation are induced in the elderly population by myeloid cells that have been exposed to external vascular stimuli. Furthermore, vascular aging worsens the atherosclerotic process, with intrinsic vascular variables significantly impairing mitochondrial function [20].

Comorbidities may worsen the changes seen in senescent cells. For both surgical and interventional revascularization techniques, the best medical therapy and risk factor management may be the sole viable strategies. Current ultrastructural research on ITA specimens on the function of age and comorbidities have significant clinical implications in emphasizing the aim for lowering the burden of these variables in elderly patients undergoing CABG.

There are some potential limitations of the study. Firstly, the concomitant variables that impact ITA morphology might also affect the morphology of target coronaries and aorta. Thus, the degree of ITA morphology does not directly connect to patency rates. Secondly, there is no control group (without coronary disease) for comparison. Finally, due to the lack of clinical follow-up results, we did not extrapolate our histopathological findings to clinical outcomes or graft patency.

In conclusion, while the ITA may experience ultrastructural alterations in elderly patients with comorbidities, it remains the gold standard graft in CABG with its native resistance to atherosclerosis.

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Conflicts of Interest

None.

Competing Interests

The author(s) declare that none

Ethics Committee Approval

This study was approved by the Institutional Review Board of Hacettepe University Hospital (IRB number 16969557-1711), and informed consent was obtained from all participants.

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