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Investigation of the histopathological differences of radial artery used for coronary bypass surgery by electron microscopy in two different age groups

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ABSTRACT

Aim: The study aims to investigate by electron microscopy whether there are any histopathological differences between the samples collected from the radial arteries used as grafts in the coronary bypass surgeries in two different age groups.

Methods: Forty patients whose radial artery grafts were prepared for myocardial revascularization purposes were included in this study. The patients were divided into two groups of twenty each: patients over and under 55. All patients included in the study were evaluated preoperatively for the arterial circulation of the hand from which the radial artery graft would be harvested. The radial artery was dissected as a pedicle together with the surrounding venous structures by means of low-voltage electrocautery. It was left in its original anatomic site until the harvesting of the sample. The harvested samples were examined using a transmission electron microscope, and their photos were taken.

Results: We found that the preoperative and intraoperative variables in both age groups were statistically similar except for age, and that the vessels in both groups were histologically normal, with minimal pathological changes.

Conclusion: We concluded that radial artery is a graft that can be used in suitable cases in coronary bypass surgery with no age restrictions.

Key words: Radial artery, coronary artery bypass surgery, electron microscopy.

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Introduction

Despite the improvements in preventive medicine since the development of cardiac surgery and people being more conscious, the age range of patients who underwent coronary artery bypass surgery (CABS) is rapidly expanding depending on the increase of coronary artery disease and spread of coronary angiography [1]. Until recently coronary artery disease was not considered even in the differential diagnosis in patients under 40, coronary artery disease and CABS patients is on the rise in patients <40 years due to factors including hard working conditions, the change in eating habits, stress, etc. [2]. In recent years, the rate of older patients undergoing CABS is gradually increasing compared with younger patients [3-4]. This increase is also associated with the improvements in cardiac anesthesia, surgical and myocardial protection techniques as well as the use of percutaneous transluminal coronary angioplasty in younger patients [5].

The long-term success of CABS is dependent on the stability and durability of grafts used [6]. Despite the successful results in CABS with an anastomosis of the left internal thoracic artery to the left anterior descending artery (LAD), problems such as graft failure in the long-term caused by SVGs used for other vessels cause various limitations [8-9].

Their easy preparation, preparation in parallel with ITA, and sufficient length as well as reports of high patency rates are making them attractive alternatives to radial artery (RA) vein grafts and non-ITA arterial grafts [9-10]. The study aims to investigate by electron microscopy whether there are any histopathological differences between the samples collected from the radial arteries used as grafts in the coronary bypass surgeries in two different age groups.

Materials and methods

Forty patients who were expected to receive myocardial revascularization were divided into two groups of twenty each as patients over and under 55 after approval of the hospital's ethical committee. Allen test was performed in all patients. The patients were excluded from the study if they suffered from Raynaud's phenomena and had anatomic vascular anomalies of the upper extremity, arteriovenous fistula for hemodialysis or indications of patency, a history of trauma of the arterial system of the forearm, or a previous surgery for the arterial system of the forearm. RA was removed simultaneously with LITA. RA was dissected as a pedicle together with the surrounding venous structures by means of low-voltage (25 V) electrocautery. Grafts were left in their original anatomical sites until arterial anastomosis. In this period, RA was covered with isotonic salt water and *papaverine-soaked* gauze. No strong mechanical or hydrostatic dilatation was done in order to avoid intimal damage. After measurement of graft lengths for the target coronary artery where anastomoses are planned, RA samples of 0,5mm were harvested from the proximal end of the RA graft immediately before aorta-RA graft proximal anastomosis. The process where RA samples were harvested immediately before the removal of RA from its anatomical site and the proximal anastomosis stage was recorded as the time when the samples remained ischemic.

The harvested RA samples were fixed in a 2,5% glutaraldehyde solution for 24 hours. Then the samples were diluted in a Sorenson's Phosphate Buffer solution, pH 7,4, and 1% OsO_4 post-fixation was performed. Thereafter, the samples were diluted again with SPB solution and dehydrated. The dehydration procedure was performed in alcohol concentrations (25%, 50%, 75%, and pure alcohol) varying from low to high, then the samples were diluted twice with propylene oxide, and *preparations for the mounting procedure started*. At the initial step of the mounting procedure, 1:1 propylene oxide and epoxy resin mounting material were mixed and the samples were kept in this mixture for 1 hour; at the end of 1 hour, same amount of epoxy resin mounting material is added into this mixture to make the mixture ratio 1:3. Following this procedure, the samples were mixed in a rotator for 1 night and mounting preparations were thus terminated. Thereafter, the samples were mounted into the epoxy resin mounting material using plastic capsules were kept in a drying oven at 60ºC for 48 hours. At the end of 48 hours, the samples were removed from the drying oven and semi-thin sections of the samples were cut by means of an ultramicrotome. At a thickness of 2 micrometers, these sections were stained with methylene blue and observed by light microscopy to determine the optimum thin-sectioning areas. Transmission electron microscopy section tissue surface dimensions were obtained by trimming the tissue surface of the optimum thin-sectioning areas. The thin-sections of the samples at about 60 nanometers thick were cut using the same ultramicrotome. After staining of the thin-sections by double contrast staining with uranyl acetate and lead citrate, they were examined by transmission electron microscopy and their photos were taken.

The descriptive statistics of analysis variables are given in Table 1. ANOVA and various other tests were used to determine whether or not intragroup differences were statistically meaningful. Table 2 shows the Levene Statistics and the results of tests conducted to determine whether the variances of the used variables were different between the two groups. This test is important as the ANOVA analysis shows no variance differences between the two groups. On the other hand, Table 3 shows the results of the Kolmogorov-Smirnov test which shows whether the variables have a normal distribution. Test results show that the normality assumption was met for all variables.

Table 1. Descriptive statistics for continuous variables by groups and for the complete study population.

Variable	Group	N	Mean ± Std. Deviation	Minimum	Maximum
Age	Over 55 years	20	64.65 ± 7.65	58	80
	Under 55 years	20	50.75 ± 4.61	37	55
	All	40	57.7 ± 9.4	37	80
	All	40	0.48 ± 0.51	0	1
Cholesterol	Over 55 years	20	172.25 ± 35.48	134	257
	Under 55 years	20	176.9 ± 37.48	106	239
	All	40	174.58 ± 36.1	106	257
HDL	Over 55 years	20	35.1 ± 8.01	23	54
	Under 55 years	20	34.45 ± 5.38	26	47
	All	40	34.78 ± 6.74	23	54
LDL	Over 55 years	20	97.9 ± 37.47	51	202
	Under 55 years	20	98.75 ± 37.52	13	157
	All	40	98.33 ± 37.01	13	202
Urea	Over 55 years	20	40.85 ± 16.57	13	95
	Under 55 years	20	35.4 ± 9.54	23	55
	All	40	38.13 ± 13.63	13	95
CPB Time	Over 55 years	20	94.05 ± 21.01	56	139
	Under 55 years	20	88.75 ± 25.48	43	152
	All	40	91.4 ± 23.21	43	152
XCL Time	Over 55 years	20	54.1 ± 11.91	24	69
	Under 55 years	20	50.8 ± 11.09	29	82
	All	40	52.45 ± 11.48	24	82
Ischemia Time	Over 55 years	20	59.3 ± 19.8	36	120
	Under 55 years	20	53.4 ± 15.01	20	75
	All	40	56.35 ± 17.599	20	120

Results

75% of Group 1 (over 55 years old) and all of Group 2 were male patients. While the mean age of all patients in Group 1 was around 64, patients aged around 50 in Group 2. 55% of patients in Group 1 and 40% of patients in Group 2 were hypertensive. The mean cholesterol and urea values and smoking habits as well as the standard deviations of the two groups were close to each other. There were no meaningful differences in CPB, XCL and ischemia times.

Table 2. Variance Homogeneity test.

Variable	Levene Statistics	1st Degree of Freedom	2nd Degree of Freedom	*p*
HT	0.35	1	38	0.56
Cholesterol	0.19	1	38	0.66
HDL	3.20	1	38	0.08
LDL	0.05	1	38	0.83
Üre	1.24	1	38	0.27
DM	17.45	1	38	0.00
Smoking	0.55	1	38	0.46
CPB Time	0.13	1	38	0.72
XCL Time	0.05	1	38	0.83
Ischemia Time	0.29	1	38	0.60

Table 3. Kolmogorov - Smirnov test.

Variable	Kolmogorov Smirnov Statistics	*p*
Cholesterol	0.59	0.87
HDL	0.61	0.86
LDL	0.64	0.80
Urea	0.75	0.64
CPB Time	0.56	0.92
XCL Time	0.71	0.70
Ischemia Time	0.75	0.64

Ultrastructural examinations of RA samples of all age groups by transmission electron microscopy showed focal minimal ischemic changes. Vessels in both groups were observed to be histologically normal, with minimal pathological changes. Small vacuoles were found in the endothelial cells in the intima layer. Endothelial cells where small vacuoles were found were present in the group over and under 55 years old.

The basal membrane, subendothelial layer, and internal elastic membrane were ultrastructurally normal in all samples. An electron microscopy evaluation of the tunica media layer showed normal smooth muscle cells in all samples. The external elastic membrane on the outer part of the tunica media was observed to be normal in all samples. An electron microscopy evaluation of the tunica adventitia layer, the samples were found to have collagen fibers that were ultrastructurally normal; however, there were small edematous areas between the collagen fibers.

The connective tissue cells in the tunica adventitia were examined to be ultrastructurally normal. And there were dilatations in small vacuoles and perinuclear cisterns in the cytoplasm of fibroblasts. As a result, minimal focal pathological changes were found between the fibroblasts and collagen fibers in the endothelial cells of the RA samples in both groups (image1-2-3-4). We assumed that such focal minimal pathological reversible changes found in the samples were due to the time period between the vascular dissection and fixation. Furthermore, these changes did not differ between the two groups and had no statistical meanings, all being reversible changes.

Table 4. ANOVA results.

Variables		Total Squares	Degree of Freedom	Mean Squares	F Statistics	*P*
HT	Inter-group	0.23	1	0.23	0.88	0.35
	Intra-group	9.75	38	0.26		
	Total	9.98	39			
Cholesterol	Inter-group	216.23	1	216.23	0.16	0.69
	Intra-group	50615.55	38	1331.99		
	Total	50831.78	39			
HDL	Inter-group	4.22	1	4.22	0.09	0.76
	Intra-group	1768.75	38	46.55		
	Total	1772.98	39			
LDL	Inter-group	7.22	1	7.22	0.01	0.94
	Intra-group	53415.55	38	1405.67		
	Total	53422.78	39			
Urea	Inter-group	297.03	1	297.03	1.62	0.21
	Intra-group	6947.35	38	182.83		
	Total	7244.38	39			
DM	Inter-group	0.40	1	0.40	3.23	0.08
	Intra-group	4.70	38	0.12		
	Total	5.10	39			
Smoking	Inter-group	0.03	1	0.03	0.14	0.71
	Intra-group	6.95	38	0.18		
	Total	6.98	39			
CPB Time	Inter-group	280.90	1	280.90	0.51	0.48
	Intra-group	20726.70	38	545.44		
	Total	21007.60	39			
XCL Time	Inter-group	108.90	1	108.90	0.82	0.37
	Intra-group	5029.00	38	132.34		
	Total	5137.90	39			
Ischemia Time	Inter-group	348.10	1	348.10	1.13	0.29
	Intra-group	11731.00	38	308.71		
	Total	12079.10	39			

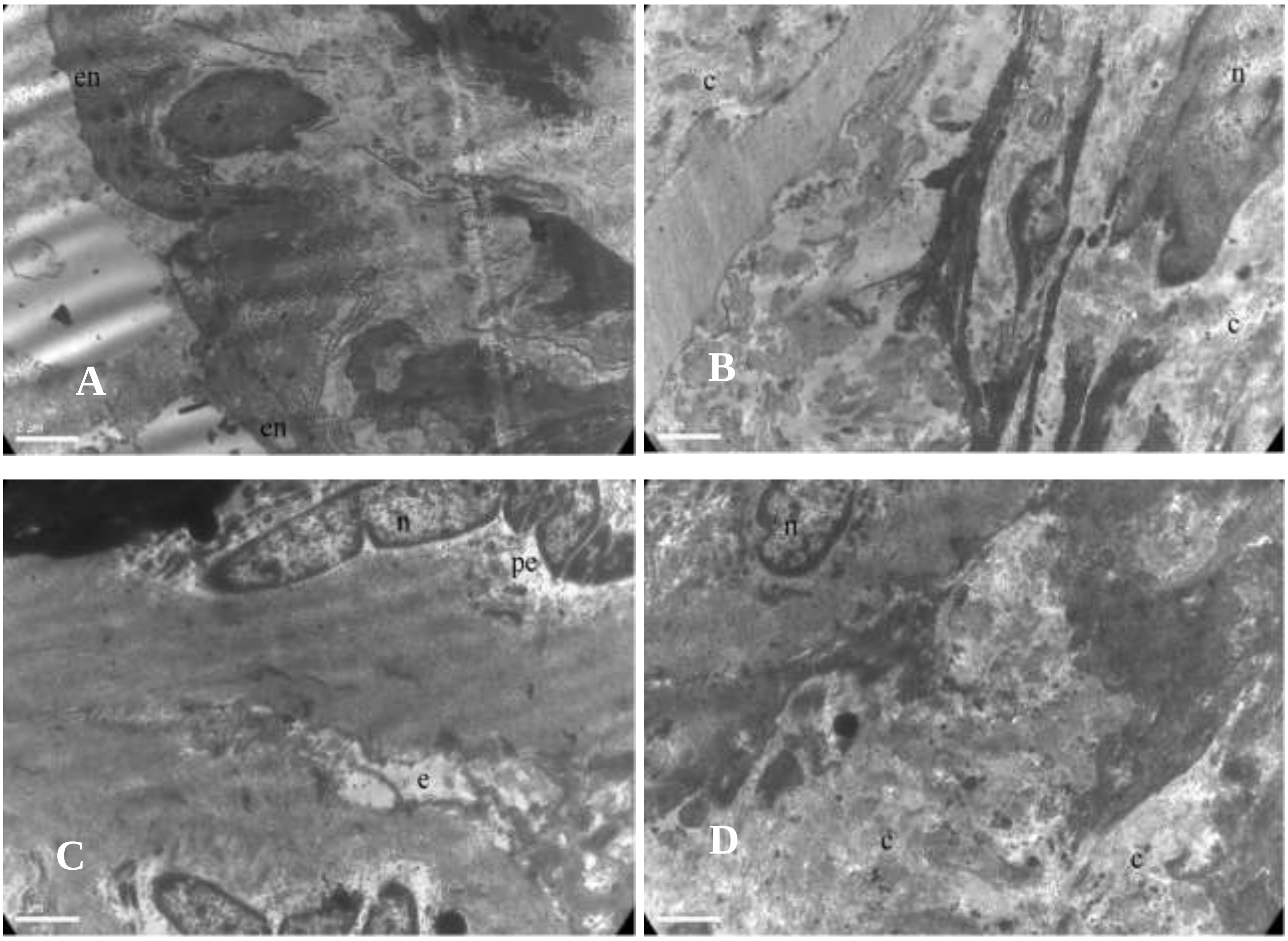

Figure 1. A) Electron microscopy (EM) view of an endothelial cell of a patient over 55. **B)** EM view of an endothelial cell of a patient under 55. **C)** EM view of the adventitia of a patient under 55. **D)** EM view of the adventitia of a patient over 55.

Discussion

Carpentier described the use of RA in coronary artery surgery in 1971. Several years later, he abandoned this grafting due to occlusion at an early stage [11]. In the 1990s, when the concept of total arterial myocardial revascularization has become popular, an unexpected finding whereby the RA grafts placed 20 years ago were demonstrated to be still patent on angiography raised new enthusiasm for RA as an alternative arterial conduit.

Angiographic studies conducted at 6-36 months postoperatively report the RA graft medium-term mean patency rate to be between 87.5% and 100%, and the full patency rate to be in varying rates between 64.9% and 100% [12].

In the series of Tatoulis, one of the largest series, 271 cases underwent angiography 14.4 ± 10.4 months (1 -35. months) postoperatively, and an examination of 280 RA grafts, and 369 RA coronary artery anastomoses showed that 36 (10%) anastomoses were either occluded or had “string signs”. 333 (90.2%) of the 369 anastomoses were judged to be patent. The same series report the RA patency to be 90.7% and 92.7% for LAD and Cx, respectively, pointing out a reduced rate in RCA (86.7%) [10-13].

A review of the literature also showed authors with opposing views who suggest that RA is not as ideal graft as it is believed to be. Hence, Khot et al. from the Cleveland Clinic compared the medium-term patency rates of LIMA, RITA, RA and SVG. They reported that of the 398 grafts that were studied, RA was the one with the lowest overall patency, and the highest rates of severe disease and occlusion out of these four types of grafts. So, they claimed that RA was different from ITA for patency rates and it could only be used in select cases [14]. Chong et al. compared RA and saphenous graft patency rates together with flow rate and flow volume measurements and found that RA had more flow and intact endothelial functions similar to physiological coronary artery flow dynamics compared with SVG [15].

There are a limited number of available arterial conduits for complete arterial revascularization. To overcome this problem, one conduit was used for several distal anastomoses using sequential or composite graft techniques. It was found that CPB time was significantly shortened in patients who underwent composite grafting since proximal anastomoses is previously prepared and it allowed the use of a longer conduit, and that there were fewer embolic events such as cerebrovascular events due to minimized ascending aorta manipulation [16]. However, complexity and hypoperfusion potentials were also increased in this case.

This multi-centered randomized RAPS study compared RA and SVG patency rates. Aiming to find out the 5 and 10-year medium and long-term patency rates, this study currently reveals the 1-year short-term findings. In this study, while full occlusion was found in 8,2% of RA grafts in the control angiography performed at the end of 1 year, and 13,6% of SVGs. and the meaningful difference in favor of RA was eliminated where string sign is considered as full occlusion [17]. Again, in a randomized RAPCO study comparing SVG and RA grafts, a comparison of 5-year patency rates showed RA grafts to have higher patency rates compared with SVG, although not statistically significant. Since meaningful differences between patency rates of grafts generally occur after 10 years, long-term findings of this study that will soon be announced need to be seen [18].

In recent years, the rate of older patients undergoing CABS is gradually increasing compared with younger patients [3-4]. This increase is also associated with the improvements in cardiac anesthesia, surgical and myocardial protection techniques as well as the start of the use of percutaneous transluminal coronary angioplasty in younger patients [5]. About 40% of people over the age of 80 have symptomatic cardiovascular diseases, benefiting from surgery [5]. Compared with younger people, mortality and complication rates are apparently higher in this age group. As a result of longer life expectancy and declining birth rates in industrial countries, the population over the age of 65 is expected to constitute an important segment of the population in the next two decades, which suggests that the number of patients who will undergo cardiac surgeries will rise as the average age of the population raises [2-3-17]. A 13-year study by Curtis et al. shows that this rate was up from 8% to 50% [19]. Coronary artery disease is more severe in older people compared with young people, and they are generally found to have three-vessel disease, with a left main coronary stenosis rate 2 times higher than that of younger people. This is why, despite being a risk factor for mortality in old age, improvements in myocardial protection, perfusion, and surgery techniques are developments that will reduce this risk [20]. The foremost important factor affecting postoperative mortality in older patients is the use of LITA [4-5-20]. The combination of LITA with one or more saphenous veins in older people reduce mortality significantly (2.3%) compared with those using only SVG (8.2%) [5]. This is also reflected in survival and the use of LITA and other arterial graft extends survival [3-4-5].

Other benefits of using RA as a valuable graft in older patients include the use of grafting particularly in redo cases who have previously undergone coronary bypass operations with the use of saphenous veins, lower extremity venous disease in old age group, varicose veins of the lower extremities, and lymphedema in the lower extremities again seen at a higher rate in the older patient group compared with the younger patient population [21]. Previous studies demonstrate that factors including old age, female sex, obesity, and peripheral vessel disease play important roles in the development of complications in the saphenous vein graft wound site [22-23]. Particularly in diabetic old patients, there is higher risk of infection and sternal complications in leg incisions [24-27]. Again, the use of LITA as the area of proximal anastomosis in the older patient group and the ability to use RA successively in cases where the aorta is like a porcelain aorta in the atherosclerotic basin ensure that the aorta is not used as a proximal anastomosis area. It was found that CPB time was significantly shortened in patients who underwent composite grafting since proximal anastomoses is previously prepared and it allowed the use of a longer conduit, and that there were fewer embolic events such as cerebrovascular events due to minimized ascending aorta manipulation [16]. Higher rate of chronic obstructive lung disease and osteoporosis in the old age group plays an important role in the development of pulmonary and sternal complications. This is why optimal graft selection has an important influence on mortality and morbidity in the old age group. A previous study in patients over the age of 70 found the short and medium-term outcome of the usefulness and reliability of total arterial revascularization made with composite grafts to be quite good. On the other hand, Muneretto et al., in their 18-month follow-up study, found a 96% RA patency rate achievement in their 100 disease series over the age of 70 [16].

When choosing the patients included in this study, efforts were made to see the histopathological effects of the age variable over RA making sure that the variables up to the stage of harvesting of the preoperative and RA samples have similar values. We tried to demonstrate that the variables other than age were also statistically similar to each other. Our electron microscopy examinations showed that minimal transient ischemic changes occurred at the preparation stage and that the changes were not statistically meaningful as they were observed on all preparations. We observed no histopathological differences upon analysis of the RA samples that harvested from patient groups over and under the age of 55 by electron microscopy.

The main strategy that should be followed in all coronary artery patients who were expected to undergo surgical revascularization is the revascularization of all vessels. If intervention proves successful, the patient’s future life expectancy and quality of life depend completely on the fate of the conduit used for grafting. The patient has higher risks of problems associated with ischemic complications in the time period coinciding with the graft disease formation in the 5-7 years post-surgery. When no complete and arterial revascularization is made, not only the long-term survivals, but also the qualities of life of patients are negatively affected during this time period. It is also associated with increased additional costs in interventions aimed at rediagnosis and treatment. The factor that positively affects the long-term patient prognosis in an ideal coronary revascularization operation is the choice of arterial grafts in surgery applications following complete revascularization. From an angiographic perspective, preferring to use arterial grafts whose early, medium and long-term results and superiority over venous grafts are demonstrated bring no additional costs, morbidities and mortalities to the operation. The expectation in arterial revascularization is to increase conduit patency rates and extend the asymptomatic period. In an age where our life span has gradually improved and the quality of life expectations increased in older ages as well, it seems RA is the most noticeable candidate that will contribute to the realization of this ideal as a graft.

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