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Histopathological Examination of Internal Thoracic Artery and Saphenous Vein in Patients with Diabetes Mellitus: Structural Changes in Grafts Used in Bypass Surgery

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Introduction: Epidemiological studies show that cardiovascular mortality is two to three times higher in male gender and three to five times higher in women with diabetes than those without diabetes. In this study, LIMA, which we frequently use in coronary bypass surgery, and the comparison of structural differences, if any, that may affect the saphenous vein, were analyzed with samples taken from diabetic and non-diabetic patients.

Materials and Methods: Twelve diabetic and 12 non-diabetic patients who underwent coronary by-pass between 2003-2004 use of this type were examined. We evaluated the intimal thickness, medial thickness, intima-media ratio, and medial calcification of the biopsies from these grafts used during the operation.

Results: LIMA calcification were calculated as $10,84 \pm 0,98 \mu$ in diabetics and $12 \pm 2,15 \mu$ in the control ($p=0.048$). When the calcification in saphenous veins was evaluated as a percentage, it was calculated as $7.19 \pm 3,47 \mu$ in the diabetic and $7,95 \pm 1,16 \mu$ in the control ($p=0.037$). When the LIMA intima thickness was evaluated, it was calculated as $28,75 \pm 15,65 \mu$ and $26,36 \pm 24,05 \mu$ in the control ($p=0.044$). When the LIMA intima-media ratios were examined, it was calculated as $0.128 \pm 0.93 \mu$ in the diabetic group and $0.117 \pm 0.092 \mu$ in the control ($p=0.041$). Measurement of saphenous vein intimal thickness were found as $68,05 \pm 47,94 \mu$ in the diabetic group and $66,31 \pm 48,07 \mu$ in the control group ($p=0.038$). When measured as the medial thickness of the saphenous vein, it was found to be $422,18 \pm 131,7 \mu$ in the diabetic group and $465,6 \pm 146,28 \mu$ in the control ($p=0.032$).

Conclusion: We could not find any statistically significant difference between the two groups. Since we could not find a significant difference in terms of grafts between the diabetic and control groups, we can only say that postoperative factors cause premature occlusion of grafts in diabetics.

Keywords: Internal Thoracic Artery, Saphenous Vein, Diabetes Mellitus, Graft, Bypass Surgery

Introduction

Diabetes is an important cardiovascular risk factor. Epidemiological studies have shown that cardiovascular mortality is two to three times higher in men with diabetes and three to five times higher in women with diabetes (1). Many

prospective studies in both type 2 diabetics and non-diabetic individuals have shown that hyperglycemia is an undeniable cardiovascular risk factor. The effect of blood glucose on cardiovascular risk begins well before diabetic

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values (≥ 100 mg/dl) and continues to act increasingly with rising glucose values. Findings show that the postprandial blood glucose independently increases the risk of mortality (2).

The mechanism by which hyperglycemia leads to macrovascular changes is not fully known. Although the success rate of coronary angioplasty in diabetic patients is similar to those without diabetes, restenosis, and late complication rates were found to be high. Similarly, mortality, early and late mortality, and the need for reoperation after coronary artery bypass (CABG) were found to be higher in diabetic patients compared to those without diabetes (3). In the long-term follow-up and symptoms of patients undergoing CABG, the rate of graft patency used in bypass is important. Besides, the early patency rate depends on the operation technique, the severity of the pathology in the bypassed vessels, and the graft used. While the most ideal graft responds to physiological and anatomical needs, it must provide the necessary conditions for complete revascularization, be long enough to reach the distal coronary vessels, and remain open for life in the patient. Unfortunately, a graft that responds to all these conditions has not been found. The choice of the graft to be used for the operation depends on the maximum advantage that can be tolerated, the patient's age, risk factors, the urgency of the treatment, and finally the surgeon's experience.

It became popular when it was reported by Favoloro for the first time that is saphenous vein (GSV, alternately "long saphenous vein") could be used as a graft in occlusive vascular diseases (4). In the early 1970s, when the comparative studies performed with the internal thoracic artery (ITA) showed that saphenous flow was higher, its popularity increased (5). In studies

conducted up to our time, 8-12% of saphenous grafts are blocked in the early postoperative period, and at the end of the first year, it has been shown that this rate is 12-20%. After the first year, this occlusion rate drops to 2%. The 5-year occlusion is 22-30% cumulatively (6). After the 5th year, the occlusion rate increases, and 50% of the grafts are blocked in the 10th year (7). In 20 years of follow-up, occlusion has been reported as 81% in the first 5 years, 68% in the 5th-10th year, and 46% in 16-20 years (8).

Some of the important factors that determine the patency rate of the saphenous vein are the diameter of the vessel it is made, the amount of injury in the coronary vessel, and the flow rate to the distal. Consecutive by-passes are also found to remain open more than single grafts (9). Endothelial injury leads to the release of the relaxant factor and prostacyclin released from the endothelium, which leads to impairment of endothelial integrity, platelet, and leukocyte adhesion (10). Uniform medial thickening, followed by intimal thickening, may develop in the open graft. Although the pathology has not been clearly explained, it is thought to be due to the migration and proliferation of smooth muscle cells. Intimal thickening may occur with the accumulation of the extracellular matrix or the organization of the mural thrombus (10). However, intimal proliferation is caused by the metamorphosis of endothelial regeneration, the amount of endothelial injury and the effect of platelet-derived growth factor (PDGF) released from the vascular vessel wall (11). Intimal thickening is present in 75% of the grafts at the end of the 1st year and is the most common cause of graft stenosis 6-24 months after the operation (12).

When the saphenous vein is exposed to coronary blood flow during coronary bypass,

some hemodynamic changes occur. When the lower extremity veins are exposed to normal arterial pressure, they lose their elasticity and undergo some intrinsic changes to adapt to the arterial pressure. Medial thickening of the vein graft wall and changes in vasomotor functions have been detected in performed angiotensin-induced hypertension experimentally (11).

Increased wall tension is another warning for intimal hyperplasia. A few weeks after implantation, the tension of the artery wall in which it is implanted becomes equal, as a result, smooth muscle cell proliferation slows down (13). After the first year, lipid-loaded atherosclerotic plaque begins to appear (10). Atherosclerosis of the vein graft may develop in the area of intimal hyperplasia or a different region (14). In hyper cholesterol, the pressure applied to the artery during implantation and preparation facilitates lipid collection in the media of the graft (15).

The most commonly used graft in coronary bypass surgery is undoubtedly the Left Internal Thoracic Artery (LIMA). This artery, which is the second branch of the subclavian artery, first attracted attention with publications written in 1972 (16) and became indispensable in the mid-1980s with the demonstration that the patency rates were over 90% in 12-year follow-up (17). In studies with a large number of patients, it has been found that long-term patency rates are higher than venous grafts, especially in patients with good ventricular function, and higher survival and cardiac events are observed less (18).

It has been reported that smooth muscle cells of venous grafts respond more to growth factors secreted from platelets when compared with the smooth muscle cells of LIMA (19). Although pulsatile mechanical stress is a potent

mitogen for the saphenous vein, LIMA is not for smooth muscle cells (20). Prostacyclin, a LIMA vasodilator, and platelet inhibitor secrete higher amounts of venous smooth muscle cells (21). LIMA endothelial cells produce a significantly more potent vasodilator nitric oxide (NO) compared to the saphenous vein (22). For many reasons like this, LIMA has gained the right to be an indispensable graft.

In this study, LIMA, which we frequently use in coronary bypass surgery, and the comparison of structural differences, if any, that may affect the saphenous vein, with samples taken from diabetic and non-diabetic patients. For this purpose, biopsy samples were taken from saphenous vein and LIMA to evaluate intimal thickness, medial thickness, intima-media ratio, medial calcification, which are the markers of atherosclerosis (22). LIMA blood flows were also compared.

Materials and Methods

The left internal thoracic artery and saphenous veins of a total of 24 patients who were operated for coronary bypass surgery between 2003-2004 at Gazi University Faculty of Medicine, Department of Cardiovascular Surgery were examined. While 12 of the patients were receiving oral diabetic or insulin therapy for Type-II diabetes, they were operated on by consulting the internal medicine endocrinology clinic and the other 12 patients were admitted as the control (Table-1). Patients whose left ventricular ejection fraction (LVEF) was not lower than 40%, who were diagnosed with coronary disease and decided to coronary bypass by the council jointly made by Cardiology and Cardiovascular surgery clinics, were included in the study. Care was taken to ensure that parameters such as pre-operative information, age, echocardiography

findings, coronary angiography findings, drugs used, risk factors, laboratory information were close in the patients.

Biopsy materials of 0.5-1 mm from the distal end of LIMA and 1 cm from the proximal ends of the saphenous vein were fixed with formol to be used in the histopathological evaluation of atherosclerosis. It was made by the same team to ensure standardization in LIMA and saphenous vein extraction. Patients' ITA's were wrapped with papaverine gauze for 2 minutes at 37°C, 90-120 mmHg systolic, 60-80mmHg diastolic tension, and then measured by measuring blood flow for 1 minute (ml/min). Biopsy materials were blocked by embedding in paraffin after tissue follow-up. Then, 5 mm sections were prepared. Two sections were taken from each block, one of which was hemotoxylin-eosin, and the other was stained with a technique based on the Von Kossa technique, based on the deposition of silver to phosphate and carbonate compounds in the

areas where calcium accumulated and thus showing the calcification. Samba Technologies, Morphometry version 4.32 (2003) France program was used for measurements of intimal thickness, calcification, intima-media ratios, and medial wall thickness. Preparations Images were taken by Olympus BX-51 microscope, Sony 3CCD color video. After measuring intima and media thickness in 5 separate areas from each preparation, the average values were evaluated as a micron (μ).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences SPSS for Windows 14.0 software. The conformity of the variables to normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk tests). In the Kolmogorov-Smirnov test, cases, where the p-value was above 0.05, were accepted as a

Table-1. Comparison of demographic and laboratory data of diabetic and control group

Variable	Diabetics	Control	P Value
Age	70,5 $\pm$ 8,28	67,41 $\pm$ 9,02	p>0,05
HDL (mg/dl)	36,6 $\pm$ 5,12	55,33 $\pm$ 12,95	p>0, 05
LDL (mg/dl)	150,5 $\pm$ 32,2	136,58 $\pm$ 23,34	p>0,05
TG (mg/dl)	195,08 $\pm$ 28,6	214,41 $\pm$ 42,14	p>0,05
Cholesterol (mg/dl)	251,16 $\pm$ 29,7	228,75 $\pm$ 38,29	p>0,05
VLDL (mg/dl)	47,08 $\pm$ 5,1	44,41 $\pm$ 9,19	p>0,05
HbA1c	5,27 $\pm$ 0,66	3 $\pm$ 0,79	p=0,001
Hypertension	83,3% $\pm$	58,30%	p>0,05
EF (%)	57,16 $\pm$ 7,61	58,75 $\pm$ 7,91	p>0,05
BMI (kg/m ²)	22,91 $\pm$ 2,42	22,9 $\pm$ 2	p>0,05
Glu (mg/dl)	125,91 $\pm$ 11,8	87 $\pm$ 12	p<0,001
Ppbg (mg/dl)	180,58 $\pm$ 11,34	133 $\pm$ 14	p<0,001

HDL: High density lipoprotein, TG: Triglyceride, COL: Cholesterol, VLDL: Very low density lipoprotein, HbA1c: Hemoglobin A1c, EF: Ejection fraction, BMI: Body mass index, Glu: Blood glucose, PPBG: Post prandial blood glucose

Table-2. Histopatological results of diabetic group patients biopsies

No	INTIMA		MEDIA		ITA	Saphenous V	Medial Calcification	Saphenous V	ITA ml/dk	Number of Vessels
	ITA	Saphenous V	ITA	Saphenous V	i/m rate	i/m rate	ITA			
1	40,2	78,9	198,4	351	0,197	0,224	11,8	8,2	50	2
2	59,8	150,8	186	339,8	0,376	0,311	12,7	7,1	57	4
3	19,7	175,5	290,5	505,7	0,067	0,347	10,9	9,4	59	3
4	22,4	70	257,2	521,5	0,142	0,134	10,9	9,7	54	3
5	34,7	52,2	190,2	692	0,182	0,0,5	11,3	6,8	64	2
6	52,1	26,3	221,8	310,3	0,161	0,084	9,66	9,1	51	3
7	9,8	42,7	392,7	350,2	0,04	0,185	10,8	6,8	65	2
8	13,8	57,1	234,4	31 2,1	0,058	0,182	9,05	8,2	58	4
9	29,8	40,4	343,8	288,7	0,086	0,139	10,03	8,1	65	3
10	26,8	17,9	297	334	0,09	0,076	10,84	6,4	61	2
11	24,1	63,4	252,6	459	0,079	0,138	10,5	8,9	58	4
12	11,9	41,4	276,1	602	0,067	0,121	11,65	6,7	59	2
Mean	28,75	68,05	261,7	422,1	0,128	0,168	10,84	7,95	58,4	2,83
SD	15,65	47,94	62,87	131,7	0,93	0,088	0,98	1,16	4,98	0,83

SD: standart deviation, ITA: Internal thoracic artery, i/m rate: Intima-media rate

normal distribution. When a normal distribution was detected, the patient and control group Mann-Whitney U test was used when the student t-test was not performed. A Chi-square test was used to compare the two groups in categorical variables. A significant p-value was accepted as <0.05.

Results

In the evaluations made in the diabetic and control groups, LIMA calcification were calculated as $10,84 \pm 0,98 \mu$ percent in diabetics and $12 \pm 2,15 \mu$ in the control group ($p=0.048$). When the calcification in saphenous veins was evaluated as a percentage, it was calculated as $7.19 \pm 3,47 \mu$ in the diabetic group and $7,95 \pm 1,16 \mu$ in the control group ($p=0.037$). When the LIMA intima thickness was evaluated, it was calculated as $28,75 \pm 15,65 \mu$ and $26,36 \pm 24,05 \mu$ in the control group ($p=0.044$). When the LIMA intima-media ratios were examined, it was calculated as $0.128 \pm 0.93 \mu$ in the diabetic group and $0.117 \pm 0.092 \mu$ in the control ($p=0.041$).

Measurement of saphenous vein intimal thickness were found as $68,05 \pm 47,94 \mu$ in the diabetic group and $66,31 \pm 48,07 \mu$ in the control group ($p=0.038$). When measured as the medial thickness of the saphenous vein, it was found to be $422,18 \pm 131,7 \mu$ in the diabetic group and $465,6 \pm 146,28 \mu$ in the control ($p=0.032$).

Figure-1. Calcification in the intima and media layers of the saphenous vein

Evaluated at 40-fold magnification after hematoxylin-eosin staining

Table-3. Histopatological results of control group patients biopsies

No	INTIMA		MEDIA		ITA	Saphenous V	Medial Calcification	Saphenous V	ITA ml/dk	Number of Vessels
	ITA	Saphenous V	ITA	Saphenous V	i/m rate	i/m rate	ITA			
1	40,2	78,9	203,7	350,8	0,197	0,224	9,8	10,3	49	2
2	95,9	186,9	255	599,4	0,376	0,311	11,6	7,6	51	3
3	33	129,6	277,2	466,1	0,119	0,277	11,2	4,1	59	2
4	11,2	22,6	223	170	0,105	0,109	9,47	4,8	66	2
5	9,9	36,2	97,3	331,9	0,101	0,106	14,,1	9,8.	44	3
6	22,4	32,1	297	56,53	0,102	0,056	11,5	4,3	58	4
7	9,8	45,9	251	337,9	0,039	0,121	13,7	11,9	69	3
8	20,3	40,1	289,6	446,4	0,07	0,089	11,4	6,4	76	2
9	22,8	54,9	304	593,1	0,057	0,092	10,8	4,3	67	3
10	23,5	34,2	230,5	690	0,102	0,345	11,6	9,9	49	2
11	21,5	52	214	504,2	0,1	0,103	11,5	1,3	67	3
12	5,9	82,4	264,3	532	0,022	0,154	17,4	11,6	70	2
Mean	28,75	68,05	261,7	422,1	0,128	0,168	10,84	7,95	58,4	2,83
SD	15,65	47,94	62,87	131,7	0,93	0,088	0,98	1,16	4,98	0,83

SD: standart deviation, ITA: Internal thoracic artery, i/m rate: İntima-media rate

When the saphenous vein intima-media ratio was examined, it was calculated as $0.168 \pm 0.088 \mu$ in the diabetic group and $0.165 \pm 0.098 \mu$ in the control group ($p=0.0385$). When the LIMA currents (ml/min) were evaluated, it was 58.41 ± 4.98 (ml/min) in the diabetic group versus 60.41 ± 10.23 (ml/min) in the control group ($p>0.039$). Comparing the number of bypassed vessels in diabetic group $2,83 \pm 0.83$ and control $2.58 \pm 0,67$. Although there were differences

between the diabetic and control groups as a result of the examinations, no statistical difference was found.

Discussion

The appropriate graft to be used for coronary bypass in cardiac surgery has led to many studies. While cardiovascular mortality rates tend to decrease in the general population, this rate tends to increase in diabetics (23). Therefore, diabetes has special importance in cardiac surgery. In our study group, 75% of the group was using oral antidiabetic agents ($n=9$), while 25% were using insulin ($n=3$). It has been reported in many studies that diabetes is an important risk factor in the development of atherosclerotic lesions and occlusion of bypass grafts, independent of other factors. (24). It has been observed that distal coronary arteries are affected more frequently in diabetics (25). In the BARI study, it was shown that the rate of coronary bypass utilization of diabetic patients depends on the use of LIMA (26). However,

Figure-2. Media thickness in the internal thoracic artery

Evaluated with hematoxylineosin at a magnification of 40 times

bilateral use has been shown to cause an increase in sternal dehiscence and wound infections at a high rate. (27) In addition to studies stating that it is higher in women in terms of mortality (28), there are also studies arguing that there is no difference (29). In terms of age, although there was a difference between the diabetic group (mean 70.5) and the control group (mean 57.31) in our study, no statistically significant difference was observed.

Figure-3. Calcification in the intima and media layers of the saphenous vein



Evaluated after 40 times magnification with the von kossa technique

Figure-4. Measurement of intima thickness in internal thoracic artery



Evaluated by hematoxylin-eosin staining at 200 times magnification for more precise evaluation

In a study with occluded grafts, it is associated with cholesterol (29). The hypercholesterolemia mechanism that initiates, develops, and sustains vein graft atherosclerosis is still not completely explained. Accelerated development of atherosclerosis has been observed due to the increasing pressure in vein grafts. (29). In a study similar to our study, no difference was found in the saphenous vein and LIMA contraction and vasodilatation tests among the grafts used in coronary bypass surgery, but they found that arterial grafts were preserved in histopathological studies (30).

Conclusion

In our literature review, we found that ITA is an indispensable graft in coronary heart surgery, which has been confirmed by many studies and the saphenous vein is used most frequently. In the histopathological examination of our study, we could not find any statistically significant difference between the two groups. At the beginning of this study, we started with the argument that some factors are arising from the grafts themselves in early occlusion. Unfortunately, since we could not find a significant difference in terms of grafts between the diabetic and control groups, we can only say that postoperative factors cause premature occlusion of grafts in diabetics. Prospective studies with larger groups is needed to support this study.

Ethical Statement

The Gazi University Faculty of Medicine ethics committee approval was obtained for the study.

Conflicts of Interest

The author declared no conflict of interest.

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