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Morphometric characteristics of the great saphenous vein as graft for surgical myocardial revascularization in relation to sex

Морфометријске карактерестике велике сафенске вене као графта за хируршку реваскуларизацију миокарда у односу на пол

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Morphometric characteristics of the great saphenous vein as graft for surgical myocardial revascularization in relation to sex

Морфометријске карактерестике велике сафенске вене као графта за хируршку реваскуларизацију миокарда у односу на пол

SUMMARY

Introduction/Objective The significant difference in the patency of vein coronary grafts in relation to sex still does not have a clearly defined cause. Our research determines the existence of morphometric differences in the wall of the great saphenous vein, in relation to sex.

Methods The research included 268 patients classified by sex in five age groups, who underwent morphometric measurement of the wall of the great saphenous vein.

Results In our research, no significant difference was found in the total thickness of the vein wall between the sex ($p = 0.111$), nor a significant association between wall thickness, age and sex. The average thickness of the intimal layer of the vein wall was significantly higher in male subjects ($p = 0.005$) and multivariate regression analysis found a significant correlation between intimal thickness, age and sex ($p < 0.001$). The medial layer of the vein in women was significantly larger ($p < 0.001$), both overall and in all age groups. Multivariate regression analysis confirmed a significant association between media thickness, age and sex ($p < 0.001$). The thickness of the adventitial layer was significantly higher in men ($p = 0.031$) and a significant association between the thickness of the adventitia, age and sex was also determined ($p < 0.001$).

Conclusion Our results indicate significant morphometric differences in the wall of the great saphenous vein in relation to the sex and age of the patients, which can be related to a significant differences in the flow rate of vein grafts in coronary surgery in relation to sex.

Keywords: cardiac surgery; vein morphometry; patency

САЖЕТАК

Увод/Циљ Значајна разлика у проходности венских коронарних графтова у односу на пол још увек нема јасно дефинисан узрок. Наше истраживање има за циљ да утврди евентуално постојање морфометријксих разлика у зиду велике сафенске вене у односу на пол.

Методе Истраживањем је обухваћено 268 пацијената класификованих по полу у пет старосних група, којима је вршено морфометријско мерење зида велике сафенске вене.

Резултати У нашем истраживању није утврђена значајна разлика укупне дебљине зида вене између полова (p = 0,11) нити значајна повезаност између укупне дебљине зида, старости и пола. Просечна дебљина интималног слоја венског зида била је значајно већа код мушких испитаника (p = 0,005) а мултиваријантном регресионом анализом утврђена је значајна повезаност између дебљине интиме, старости и пола (p < 0,001). Медијални слој вене код жена био је значајно већи (p < 0,001), како укупно тако и у свим старосним групама. Мутиваријантна регресиона анализа је потврдила значајну повезаност између дебљине медије, старости и пола (p < 0,001). Дебљина адвентицијалног слоја била је значајно већа код мушкараца (p = 0,031), а утврђена је и значајна повезаност између дебљине адвентиције, старости и пола (p < 0,001).

Закључак Резултати нашег испитивања указују на постојање значајне разлике у зиду велике сафенске вене у односу на пол и старост испитиваних пацијената, што се може довести у везу са значајном разликом у проточности венских графтова у корорнарној хирургији у однсоу на пол.

Кључне речи: кардиохирургија; венска морфометрија; проточност

INTRODUCTION

The most common treatment for multiple coronary artery disease (CAD) is coronary artery bypass grafting (CABG) and the great saphenous vein (GSV) is the most commonly used conduit. Data indicated that in as many as 89,3% of cases GSV is used during CABG [1] although all analyzes of CABG results show significant differences in the patency of arterial versus venous grafts [2, 3]. Also, the obtained data indicate the existence of a significant difference in the early and late patency of vein grafts in relation to sex.

Analyzing the first 2054 patients who underwent CABG at the Cleveland Clinic, (USA) concludes that the female sex is the third most important operative risk in coronary surgery and 2 years after CABG women have significantly lower patency of vein grafts than men [4].

In a Dutch multicenter study after one year of follow-up from coronary revascularization, vein graft occlusion was 16.7% in women and 12.4% in men [5].

In the "Reduction in Graft Occlusion Rates (RIGOR) Study" analyzing 611 grafts, vein graft occlusion was found much more often in women (p = 0.03) and as one of the main significant risk factors for occlusion, independent of others, they state female sex (p = 0.01) [6].

There are numerous authors who state that female sex is one of the risk factors for poor results after CABG as wel as for vein graft occlusion [7, 8, 9], although those who deny it are not rare [10, 11].

The main cause of middle and late reduction of vein graft patency in coronary surgery is neointimal hyperplasia of the intimal layer. The main pathophysiological mechanism of intimal hyperplasia is the migration of smooth muscle cells (SMC) from the medial to the intimal layer of the vein, their proliferation and accumulation of extracellular matrix [12, 13]. This process begins immediately after the implantation of the vein into the arterial system, so that already after 4-6 weeks most veins develop intimal thickening by migration and proliferation of SMC from media and reduction of the lumen of the vein [14].

We analyzed the existence of differences in the histological morphometry of the GSV wall used for CABG in relation to sex and their association with the level of patency.

METHODS

The study included 268 patients at the Clinic for Cardiac Surgery, University Clinical Center Nis, 134 women and 134 men, randomized by sex in 5 age groups: Group 1 (up to 39 years of age); Group 2 (40-49 years old); Group 3 (50-59 years old); Group 4 (60-70 years old) and Group 5 (age 70 over). There were 27 men and women in all age groups, except in Group 1, were there 26 men and women.

The number of study participants was determined using program "G-power" 3.1.6.

Patients who were scheduled for at least one venous coronary bypass were included in the study. Exclusion criteria referred to patients who were treated with vein drug therapy or chemical vein ablation due to venous disease, patients with a history of thrombosis of superficial and deep venous system.

Patients included in the study were divided into groups according to age and sex, and simple randomization was achieved using computer-generated random numbers.

A 1cm long GSV sample was taken through a longitudinal skin and subcutaneous incision using a conventional technique at the level of the medial malleolus, without prior dilatation and lavage, and the perivascular tissue and fascia were sharply dissected with scissors.

Immediately after collection, the GSV samples were fixed in a 4% buffered formalin solution (immersion fixation) and molded into paraffin. The paraffin blocks were sectioned at a thickness of 4 µm, then deparaffinized, rehydrated and stained with hematoxylin and eosin (H&E), followed by standard protocols.

Analysis, measurements and photography of venous preparations were performed by histologists on an Olympus BX50 microscope (Olympus, Japan) equipped with a Leica DFC295 digital camera (Leica Microsystems). Morphometric analysis was performed in the "Image J" program.

Determination of the thickness of the individual layers of the vein wall and the total thickness of the vein wall was performed by measuring each preparation at tri equal distances (0º, 120º, 240º) due to large differences in the thickness of the individual layers of the same vein. The mean value of three measurements for each preparation was used for statistical processing.

The results of the statistical analysis are presented in tabular and graphical form. Calculations were performed using the SPSS program in version 18,0. (SPSS Inc., Chicago, Ill.). In all analyses, the default error of estimation is less than 0,05 or 5% as the limit of statistical significance. The Shapiro-Wilk test was used to test the normality of the distribution of numerical values. The comparison of the thickness of the tunica intima, media, adventitia, as well as the total thickness of the vein wall between men and women was performed by the Student`s t test for two independent samples. The comparison of the mean values of the mentioned characteristics between the five age groups was performed by One-Way ANOVA and subsequent Tukey post hoc test. Multivariate linear regression analysis using the enter method was used to assess the association of age and sex with the thickness of the tunica intima, media, adventitia, as well as the total thickness of the vein wall.

The study was carried out with the consent of the Ethics Committee of the Faculty of Medicine of the University of Nis, No.12-10580-2/5 from October 9, 2018, and the Ethical Committee of the Niš Clinical Centre, No.35933/8 from November 6, 2018. Prior to engaging in the research, all participants signed informed consent according to the Declaration of Helsinki.

RESULTS

The average age of all subjects in the study was 54.42 ± 14.45 years. The average age of examined women was higher than men, but without statistical significance. Also, there was no statistically significant difference in average age between sex within the same age groups.

The total average thickness of the tunica intima was significantly thicker in men (Table 1; Figure 1). The overall average thickness of the intima regardless of sex was the highest in Group 4 and it is a significantly higher value compared to Group 1 and Group 2. The total average thickness of the intima in Group 5 is significantly higher compared to Group 1.

In the examined men, the average thickness of the intima was significantly higher in Group 4 compared to all other age groups.

In the examined women, the total average thickness of the intima was highest in Group 5 and lowest in Group 1. The thickness of the intimal layer in women in Group 1 was significantly smaller compared to Group 2, 3 and 5.

Regression analysis comfirmed a significant association between intimal thickness, age and sex. Each year of age was associated with an increase in intimal thickness of 1,059µm ($p<0.001$).

In Figure 2 the trend of an increase in the thickness of the intimal layer with aging can be clearly seen, where it is somewhat more pronounced in men than in women.

The total average thickness of the tunica media of all study participants was 394.82 ± 104.10µm (Table 2; Figure 3). The total average thickness of the medial layer in women was significantly higer than in men ($p<0.001$). Comparing the average thickness of the medial layer in relation to sex, it was significantly higer in women in all age groups compared to the same male age groups.

In men, the average thickness of the medial layer was the higest in Group 2, and significantly higer compared to Group 4 and 5.

In the examined women, the average thickness of the media was the higest in Group 2 and the lowest in the Group 5. Media thickness in Group 5 was significantly smaller than Group 1, 2 and 3. Media thickness in Group 4 was significantly lower than in Group 1 and 2.

Regression analysis confirmed a significant association between the thickness of the medial layer of the vein wall, age and sex. Each year of age was associated with a decreased in media thickness of 2.411μm ($p<0.001$). In examinated women, the thickness of the medial layer was 78.217μm ($p<0.001$) higher than in men.

Figure 4 clearly shows the existence of a trend of medial thicness decline with aging, and that this decline is more pronounced in women than in men.

The total average thickness of the tunica adventitia in all subjects was 309.37 ± 104.81μm (Table 3; Figure 5). The average thickness of the adventitia was significantly higher in all examined men than in women ($p=0.031$). Compared to age groups, the thickness of the adventitia in men compared to women was significantly higher in Group 1 and 4. The overall average thickness of the adventitia of all subjects in Group 1 was significantly lower than in all other age groups. In the examined men, the average thickness of the adventitia in Group 1 was significantly lower than in Group 2, 3 and 4. In women, the thickness of the adventitia in Group 1 was significantly lower than in all other age groups. There were no significant differences between the other age groups of women.

Regression analysis confirmed a significant association between adventitia thickness, age and sex. Each year of age was associated with an increase in adventitia thickness of 1.882μm ($p<0.001$). In examined women, the thickness of the adventitia was 28.441μm ($p=0.022$) less than in men. In Figure 6, it can be seen that the trend of increasing the thickness of the adventitia with aging is somewhat more pronounced in women than in men.

The average total wall thickness in all subjects was 805.28 ± 138.31μm (Table 4, Figure 7and 8). The average total thickness of the wall in all examined women was higher than in all men, but not statistically significant ($p=0.111$). The total thickness of the wall was significantly higher in men than in women in Group 4 ($p=0.002$), while the total thickness of the wall was significantly higher in women than in men in Group 2 ($p=0.033$). Differences between total wall thickness in men and women in other age groups are not statistically significant.

Regression analysis did not confirm a significant relationship between total wall thickness, age and sex, and Figure 9 shows that there is no trend of changes in the value of total wall thickness associated with aging, both in men and in women.

DISCUSSION

In general, a very small number of studies, which in an attempt to explain the difference in the flow rate of vein grafts in relation to sex, analyzed the morphometric structure of the vein wall, especially microscopically analyzing the morphometry of the wall and its elements.

Numerous authors in earlier studies explained the significant difference in the flow rate of vein grafts in women compared to men by the smaller average diameter of the recipient coronary arteries [4, 7]. However, comparing body surface area and body mass index (BMI) with the diameter of recipient coronary arteries, it was determined that there is no difference in relation to gender, that is, that BMI is proportional to the diameter of coronary blood vessels regardless of sex [14].

In our study, the average age of the patients is significantly lower (54.42 years)

compared to the analyzed studies that examined the morphometry of the venous conduit (60 years – Human [15] and 62.9 years – Perek [16], but this data cannot be taken into consideration because we are talking about a selected and not a total group of operated patients. Otherwise, the average age of those operated on in our region [17] does not deviate significantly from the data in the literature.

In the morphometric analyzes of veins used as coronary conduits, the transverse diameter of the veins, wall diameter, and lumen diameter were most often measured, and the results were presented descriptively, and poor postoperative results were associated with veins with a thick wall and a larger lumen [7, 18]. Studies that histomorphometrically determine the diameter of the vein wall and its layers are extremely rare, especially those that compare the structure of the veins in relation to sex.

Unlike us, Human found a significant difference in the average thickness of the vein wall in men (476.7 µm) compared to women (396.2 µm) [15]. In Perek's study significantly greater thickness of the vein wall was observed in women (359.1 µm : 469.3 µm) [16]. The significant difference in the thickness of the vein wall obtained in our study compared to earlier research can be explained by the difference in the vein treatment methodology when taking the sample. Namely, in our study, a native preparation was taken, without prior dilation and stretching, unlike other studies where the veins were dilated using different techniques before fixation, and it should be noted that due to the elastic properties of the vein wall, even very small differences in pressure can result in significant differences in diameters of both the lumen and the thickness of the vein wall.

By measuring the intimal layer of the vein wall, a significant difference was found in favor of men (p=0.005), but, analyzing age groups in relation to sex, this difference is not linear. Regression analysis revealed a significant relationship between the thickness of the intimal layer, age and sex. Our results correlate with Human's, which also found a significantly thicker intima in men [15].

The average thickness of the medial layer of the vein wall of all participants was 394.82 ± 104.10μm, with a significantly higher thickness found in women, both in total (388.42 ± 100.55:311.22 ± 92.98μm; p < 0.001), as well as in relation to all age groups. Regression analysis determined a significant association between medial thickness, age and sex, where each year of age is associated with a decrease in medial thickness by 2.411μm (p < 0.001), and the trend of decreasing medial layer thickness is more pronounced in women. In the mentioned study by Perek, a significantly thicker medial layer of the vein wall was observed in the group of patients with an occluded or severely damaged venous conduit (257.2:211.5μm; p < 0.001), and the "Logistic Regression Model" indicates that hypertrophy of the medial layer represents one of the independent risk factors for the development of severe graft disease and poor outcome after coronary surgery in patients with venous aortocoronary bypass [16].

All previous findings have confirmed that the main reservoir of smooth muscle cells (SMC) is precisely in the medial layer of the vein wall. Proliferation and migration into the intimal layer of these SMCs is the main cause of intermediate and late reduced flow or occlusion of the vein graft in coronary surgery [12, 13]. Comparing our and Perek's results, as well as the mentioned facts, it can be concluded that there is a direct correlation of the thickness of the medial layer with the results in coronary surgery, i.e., considering the results of our research, that the greater thickness of the medial layer of the vein wall found in women can be the cause of a significant difference in patency of vein grafts in women. From both a public health and clinical perspective, our results yield the importance of promoting timely cardiovascular prevention in women who – regrettably – like in other more developed countries present later for any kind of revascularization, with a greater burden of the disease and are often mis-diagnosed due to our cultural regional habits [19]

CONCLUSION

Unfortunately, there are very few histological morphometric studies of venous conduits in coronary surgery, especially comparative in relation to sex that we could compare with our results.

More research is needed to determine the true causes of the different patency of vein grafts by sex.

Our results may point to the conclusion that in women, especially younger and middle-aged women, due to significantly thicker tunica media, the use of venous conduit in coronary surgery should be avoided due to better, both medium-term and long-term, postoperative results.

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Conflict of interest: None declared.

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Table 1. Thickness of tunica intima (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	X	153.85	96.04	124.95	< 0.001
	SD	58.67	38.75	57.23	
	Med	150.7	93.37	106.43	
	Min	30.92	24.87	24.87	
	Max	288.97	236.24	288.97	
Group 2	X	110.97	151.74	131.36	0.002
	SD	41.22	49.77	49.72	
	Med	102.13	159.41	124.25	
	Min	14.19	43.65	14.19	
	Max	193.88	248.87	248.87	
Group 3	X	148.95	152.63	150.79	0.818
	SD	59.51	57.07	57.78	
	Med	138.59	139.54	139.23	
	Min	59.32	73.90	59.32	
	Max	354.05	297.64	354.05	
Group 4	X	208.4	129.81	169.1	< 0.001
	SD	50.53	46.6	62.38	
	Med	200.36	113.31	177.82	
	Min	84.35	63.12	63.12	
	Max	314.95	214.54	314.95	
Group 5	X	164.7	153.45	159.08	0.500
	SD	48.32	71.27	60.58	
	Med	164.42	146.80	157.52	
	Min	76.69	12.62	12.62	
	Max	278.82	383.79	383.79	
Total	X	157.4	137.04	147.22	0.005
	SD	60.17	57.51	59.63	
	Med	154.61	126.94	139.23	
	Min	14.19	12.62	12.62	
	Max	354.05	383.79	383.79	
Comparison between age groups		A*, C†, F‡, G†, H‡, J*	A†, B†, D†	C†, D*, F*	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max –maximum; A – Group 1 *vs.* Group 2; B – Group 1 *vs.* Group 3; C – Group 1 *vs.* Group 4; D –Group 1 *vs.* Group 5; E – Group 2 *vs.* Group 3; F – Group 2 *vs.* Group 4; G – Group 2 *vs.* Group 5; H – Group 3 *vs.* Group 4; I – Group 3 *vs.* Group 5; J – Group 4 *vs.* Group 5;

*p < 0.05

†p<0.01;

‡p < 0.001

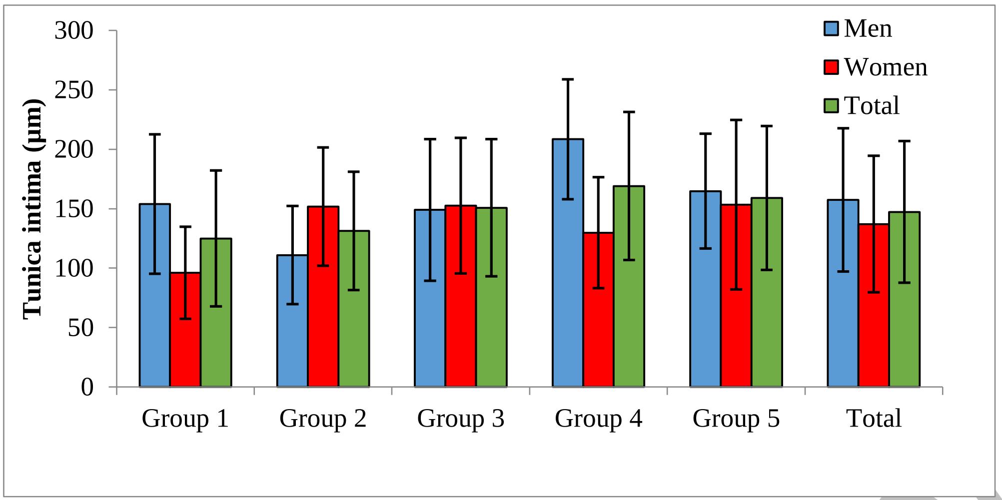

Figure 1. Comparison of intimal thickness (µm) by sex and age

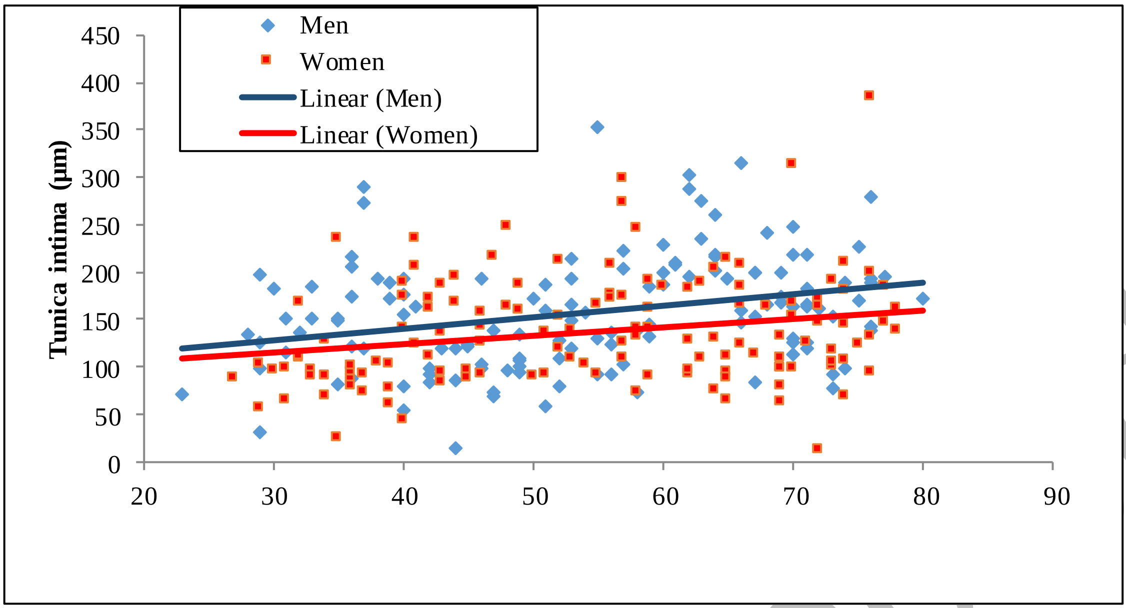

Figure 2. Correlation between the thickness of the intimal layer and age

Table 2. Thickness of tunica media (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	X	319.7	430.36	375.03	< 0.001
	SD	52.74	104.85	99.37	
	Med	314.88	413.79	365.42	
	Min	191.45	164.71	164.71	
	Max	422.86	721.67	721.67	
Group 2	X	361.34	448.82	405.08	0.005
	SD	132.01	79.73	116.69	
	Med	376.4	435.52	409.67	
	Min	126.93	265.3	126.93	
	Max	781.07	593.51	781.07	
Group 3	X	328.02	398.32	363.17	0.003
	SD	91.91	75.92	90.72	
	Med	319.21	384.19	369.44	
	Min	178.64	221.48	178.64	
	Max	512.99	601.65	601.65	
Group 4	X	291.88	361.96	326.92	0.006
	SD	81.99	98.62	96.54	
	Med	275.51	361.22	317.61	
	Min	164.89	147.33	147.33	
	Max	503.79	546.61	546.61	
Group 5	X	255.5	304.17	279.84	0.006
	SD	48.64	74.52	66.99	
	Med	267.36	300.84	278.9	
	Min	164.04	150.32	150.32	
	Max	332.89	448.96	448.96	
Total	X	311.22	388.42	349.82	< 0.001
	SD	92.98	100.55	104.1	
	Med	300.61	388.25	344.64	
	Min	126.93	147.33	126.93	
	Max	781.07	721.67	781.07	
Comparison between age groups		F*, G‡, I*	C*, D‡, F†, G‡, I†	D‡, F‡, G‡, I‡	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max – maximum; E – Group 2 *vs.* Group 3; F – Group 2 *vs.* Group 4; G – Group 2 *vs.* Group 5; H – Group 3 *vs.* Group 4; I – Group 3 *vs.* Group 5; J – Group 4 *vs.* Group 5; C – Group 1 *vs.* Group 4; D – Group 1 *vs.* Group 5;

*$p < 0.05$;

$^{\dagger}p < 0.01$;

$^{\ddagger}p < 0.001$

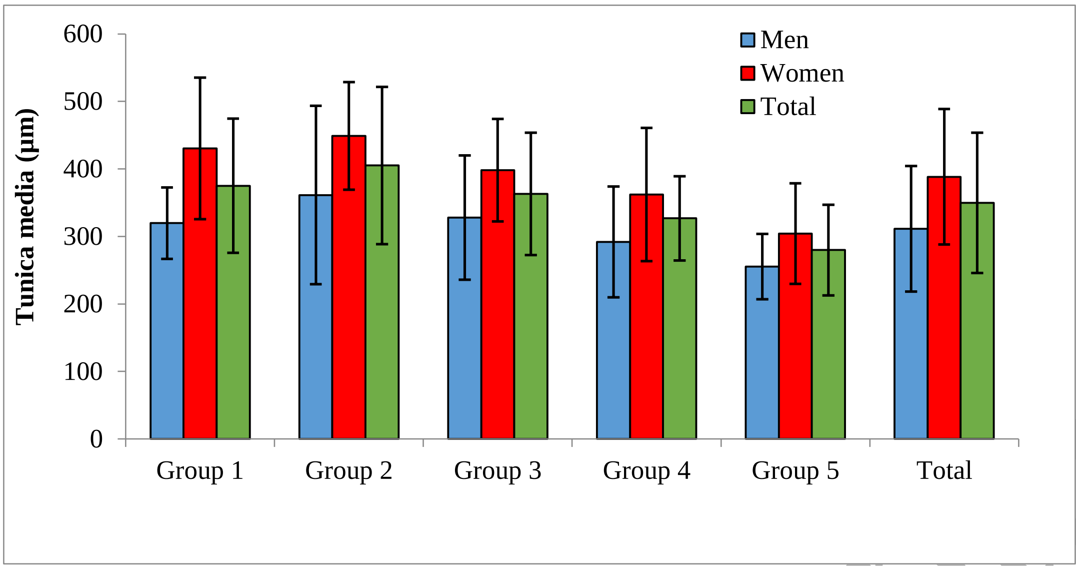

Figure 3. Comparison of tunica media thickness (µm) by sex and age

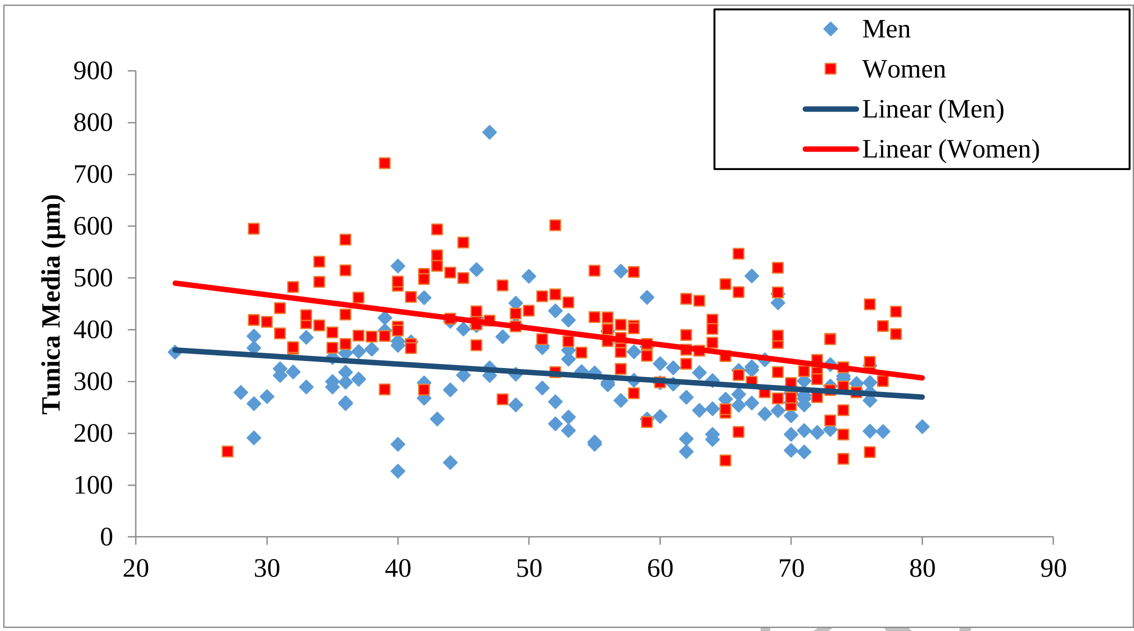

Figure 4. Correlation between the thickness of the medial layer and age

Table 3. Thickness of tunica adventitia (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	X	241.99	206.98	224.48	0.014
	SD	47.55	51.82	52.32	
	Med	264.66	201.82	236.05	
	Min	143.54	104.85	104.85	
	Max	303.25	316.08	316.08	
Group 2	X	330.75	309.5	320.13	0.553
	SD	159.14	94.42	130.05	
	Med	309.52	307.81	308.67	
	Min	167.89	157.14	157.14	
	Max	951.18	475.75	951.18	
Group 3	X	364.6	344.09	354.34	0.403
	SD	91.46	87.17	89.1	
	Med	367.96	354.86	367.42	
	Min	168.65	203.04	168.65	
	Max	511.92	560.21	560.21	
Group 4	X	360.35	276.60	318.48	0.001
	SD	62.28	104.65	95.19	
	Med	377.64	291.47	337.74	
	Min	216.45	48.19	48.19	
	Max	473.56	482.64	482.64	
Group 5	X	315.23	337.29	326.26	0.403
	SD	92.69	99.27	95.78	
	Med	301.82	347.22	328.22	
	Min	178.54	156.29	156.29	
	Max	516.01	473.42	516.01	
Total	X	323.19	295.55	309.37	0.031
	SD	106.73	101.38	104.81	
	Med	309.73	291.36	300.80	
	Min	143.54	48.19	48.19	
	Max	951.18	560.21	951.18	
Comparison between age groups		A*, B‡, C‡	A†, B‡, C*, D‡	A‡, B‡, C‡, D‡,	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max – maximum; A – Group 1 *vs.* Group 2; B – Group 1 *vs.* Group 3; C – Group 1 *vs.* Group 4; D – Group 1 *vs.* Group 5;

*p < 0.05;

†p < 0.01;

‡p < 0.001

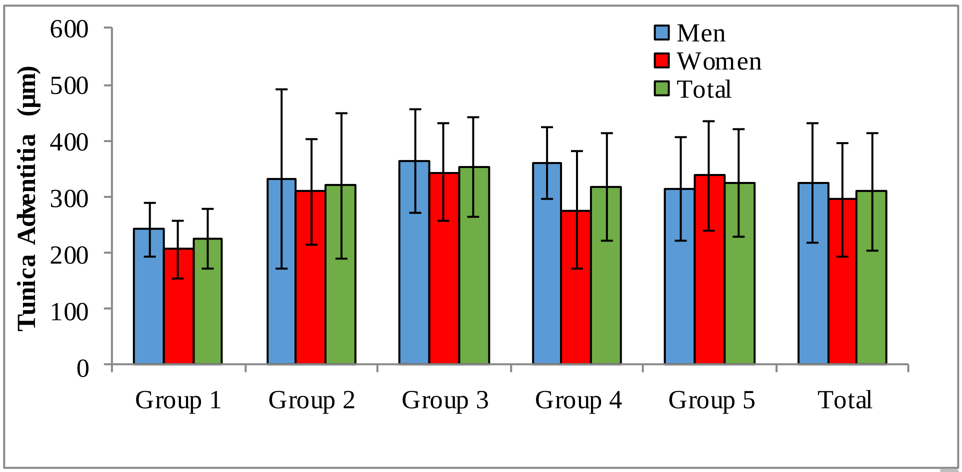

Figure 5. Comparison of tunica adventitia thickness (µm) by sex and age

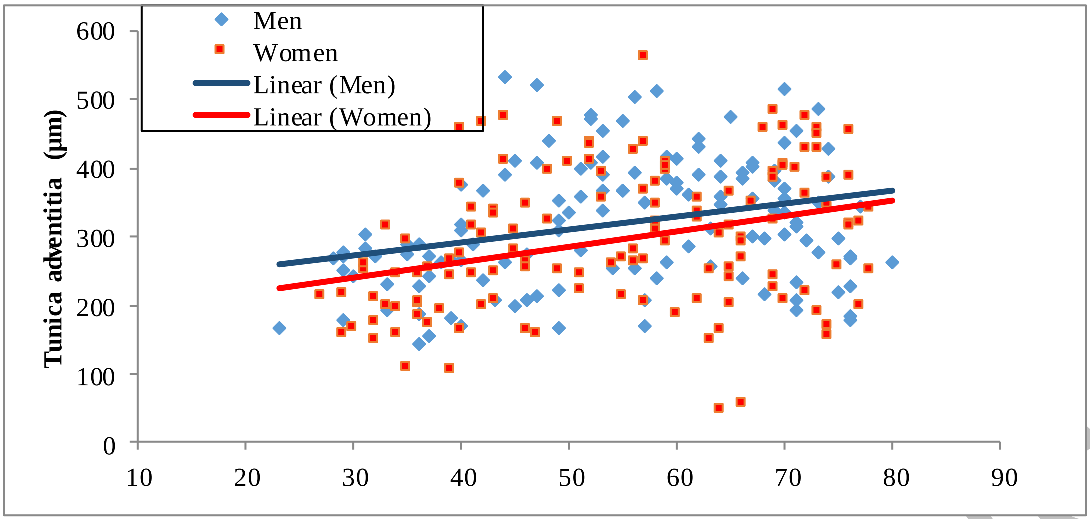

Figure 6. Correlation between the thickness of the tunica adventitia and age

Table 4. Thickness of total vein wall (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	Xsr	715.56	733.38	724.47	0.559
	SD	80.2	131.75	108.37	
	Med	708.52	717.49	714.52	
	Min	579.38	450.14	450.14	
	Max	890.08	1066.58	1066.58	
Group 2	X	803.02	898.95	850.99	0.033
	SD	185.19	131.53	166.3	
	Med	812.27	901.08	849.56	
	Min	419.98	512.95	419.98	
	Max	1346.72	1180.01	1346.72	
Group 3	X	841.57	895.03	868.3	0.054
	SD	89.87	108.49	102.3	
	Med	823.97	872.68	854.71	
	Min	675.63	716.25	675.63	
	Max	1023.05	1157.85	1157.85	
Group 4	X	860.63	768.37	814.5	0.002
	SD	89.53	115.82	112.61	
	Med	850.93	785.24	816.58	
	Min	724.99	497.65	497.65	
	Max	1059.6	950.56	1059.6	
Group 5	X	735.44	794.91	765.17	0.123
	SD	98.27	170.83	141.26	
	Med	734.68	807.99	763.24	
	Min	525.11	389	389	
	Max	910.32	1219.98	1219.98	
Total	X	791.81	818.76	805.28	0.111
	SD	127.35	147.7	138.31	
	Med	792.3	826.98	812.04	
	Min	419.98	389	389	
	Max	1346.72	1219.98	1346.72	
Comparison between age groups		B†, C‡, I†, J†	A‡, B‡, F†, G*, H†	A‡, B‡, C†, G†, I‡	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max – maximum; A – Group 1 *vs.* Group 2; B – Group 1 *vs.* Group 3; C – Group 1 *vs.* Group 4; F – Group 2 *vs.* Group 4; G – Group 2 *vs.* Group 5; H – Group 3 *vs.* Group 4; I – Group 3 *vs.* Group 5; J – Group 4 *vs.* Group 5;

*$p < 0.05$;

†$p < 0.01$;

‡$p < 0.001$

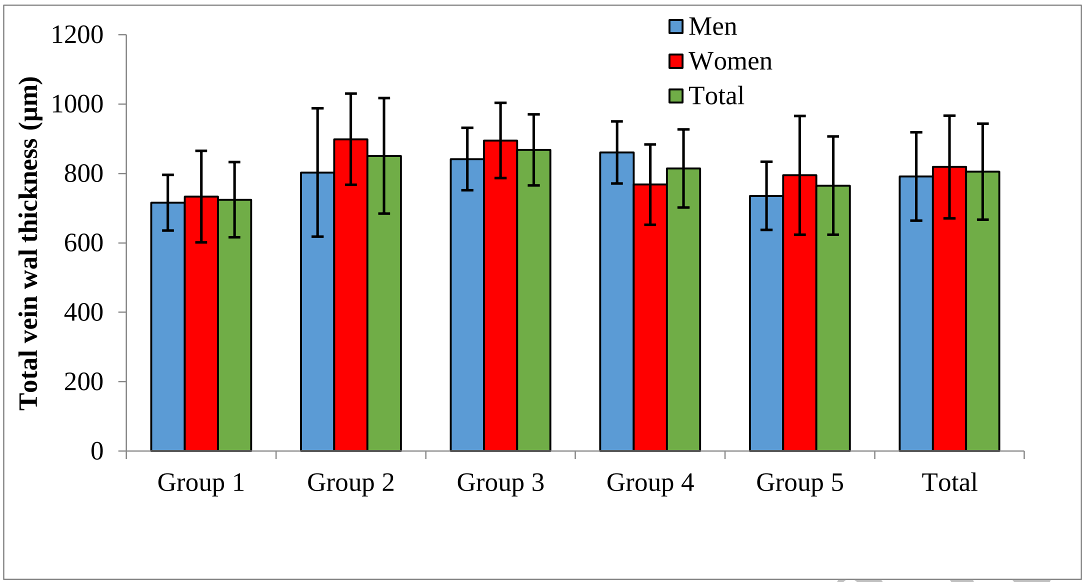

Figure 7. Comparison of total vein wall thickness (μm) by sex and age

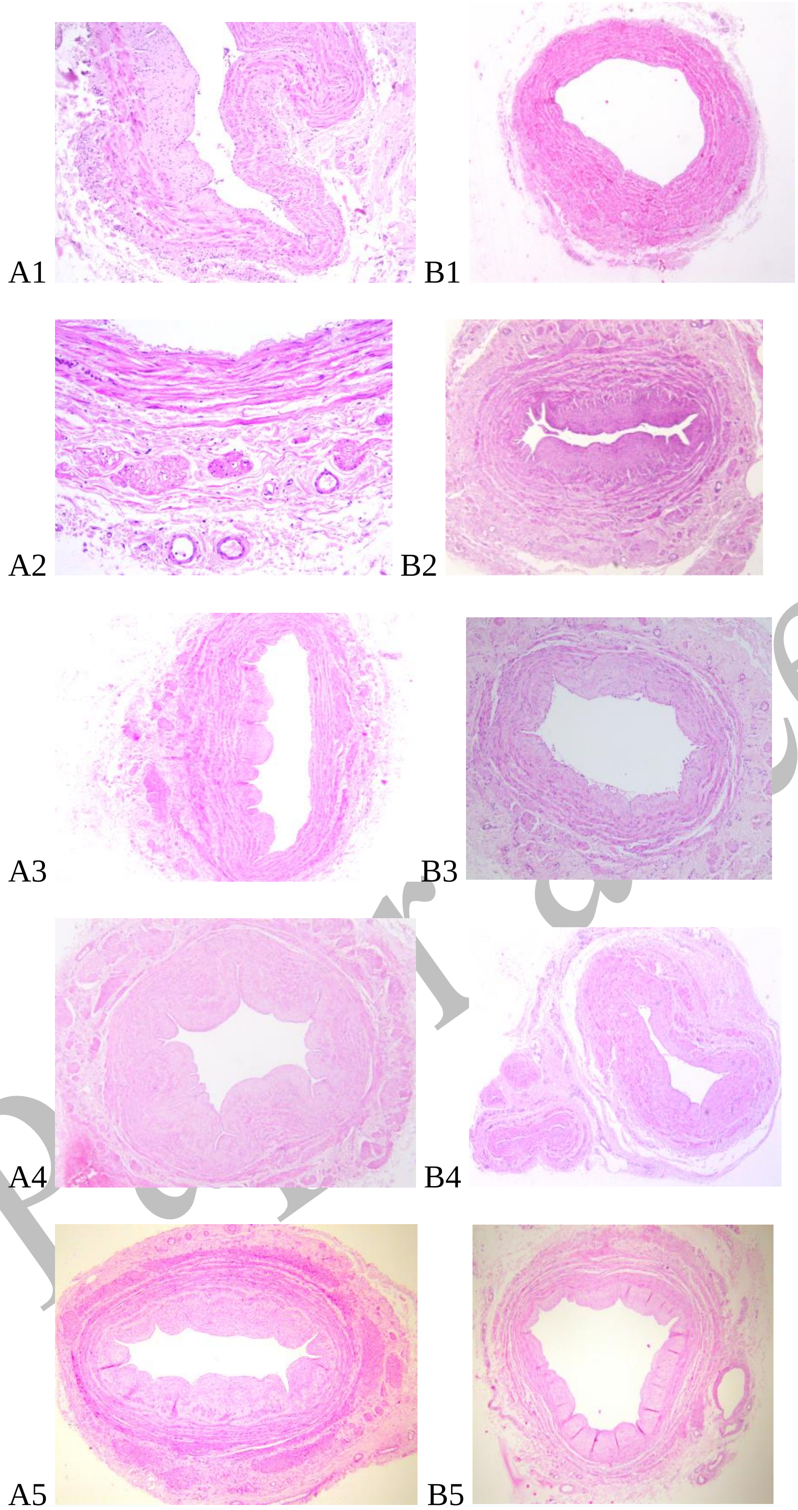

Figure 8. Cross sections of veins by sex and age groups, stained with the H&E method; A – male group [A1 – Group 1 (magnification: × 100); A2 – Group 2 (magnification: × 200); A3 – Group 3 (magnification: × 40); A4 – Group 4 (magnification: × 40); A5 – Group 5 (magnification: × 64)]; B – female group [B1 – Group 1(magnification: × 40); B2 – Group 2 (magnification: × 50); B3 – Group 3 (magnification: × 40); B4 – Group 4 (magnification: × 40); B5 – Group 5 (magnification: × 40)]

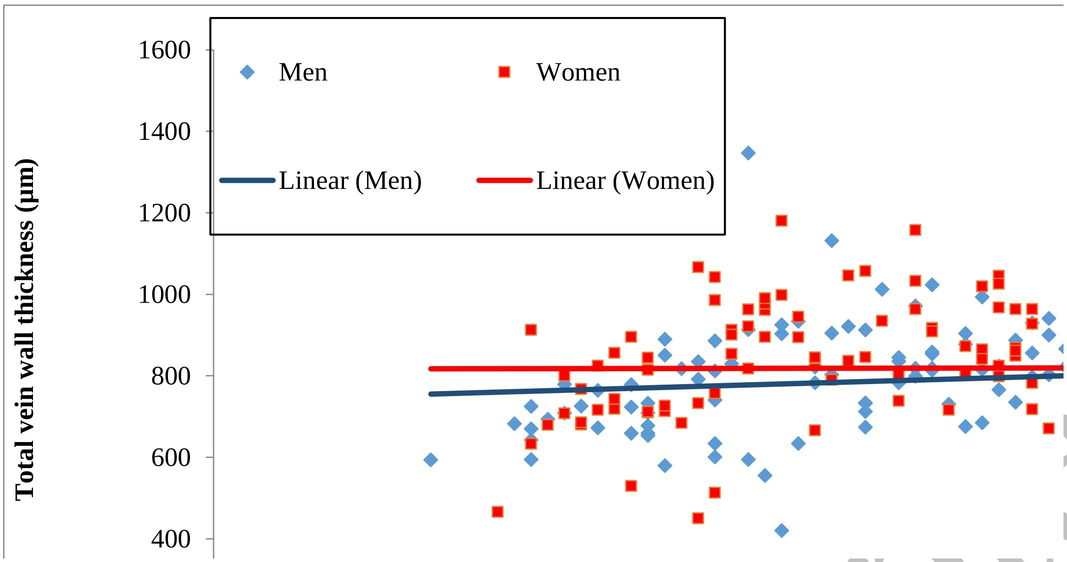

Figure 9. Correlation between total vein wall thickness and age

