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**THE ASSESSMENT OF THE ARTERIAL WALL HISTOMETRIC PARAMETERS CORRELATION
WITH THE CLASSICAL RISK FACTORS FOR CARDIOVASCULAR DISEASES (CVD) / ARTERIAL
HISTOMETRIC PARAMETERS CORRELATION WITH RISK FACTORS FOR CVD**

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Abstract

The research aim: to study the features of morphometric parameters of the arterial wall and their correlation with risk factors for the cardiovascular diseases. Methods: there were examined the carotid arteries of 106 deceased people aged 19 to 95 years without cardiovascular diseases detected during life or autopsy. There were two age groups: conditionally younger (men up to 45 years and women up to 55 years) and elder. The following morphometric parameters were measured: external (D_{ex}) and internal diameters (D_{in}), cross-sectional area of the arterial wall (S_{sec}), area of the

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arterial wall by external (S_{Dex}) and internal (S_{Din}) diameters, thickness of the intima-media complex (IMT). The presence and area of the atherosclerotic lesion were assessed, and the Vogenvort (W) and Kernoghan (K) indexes were calculated.

Keywords

Morphometry – Arterial wall – Atherosclerosis – Arteriosclerosis – Aging

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Introduction

The cardiovascular diseases are one of the leading causes of worldwide death¹. Therefore, the issue of their prevention remains one of the most acute in the medicine. The one of the main risk factors for CVD is an age that leads to the irreversible changes in the arterial wall. However, the final understanding of changes has not yet been achieved in the arterial wall during aging. It is believed that the arterial wall undergoes metabolic and morphological changes with age that contributes to the development of CVD. The entire arterial wall is subject to age variability including the intima, media and adventitia. The main signs of aging are arteriosclerosis and atherosclerosis, which is manifested by thickening of the intima and the presence of the atherosclerotic plaques. The presence of plaques can be considered as the final stage of diffuse thickening of the wall and develops as an independent pathological process. In addition to age, the lifestyle has a great influence on changes in the vascular wall. Besides age, the following risk factors are identified: dyslipidemia, tobacco Smoking, hypertension, obesity, and diabetes mellitus. Unfortunately there is currently no definitive answer to the question of whether atherosclerotic plaques are considered to be an exclusively age-related manifestation or a disease caused by prolonged exposure to the above factors².

The atherosclerosis and arteriosclerosis are certainly two different processes that have certain similarities in development, morphology and consequences. The main differences include the following: arteriosclerosis more often affects the media of the vascular wall, while atherosclerosis – intima, arteriosclerosis is diffuse, atherosclerosis is focal, the lumen of the arteries in atherosclerosis narrows, and arteriosclerosis – without changes. In addition, it is noted that more often the localization of arteriosclerosis is the aorta, while atherosclerosis more often affects carotid and coronary vessels³.

The structural changes in the vascular wall firstly develop without any clinical manifestations of CVD. At the same time the processes of arteriosclerosis and atherosclerosis take place at different speeds in each individual person. Pathological reconstruction of the arteries can begin to develop at a young age. In men up to 45 years and women up to 55 years, you can find changes in the vascular wall, which are more typical for elder people. Against the background of age-related changes, these risk factors contribute to the development of the atherosclerotic plaques. At the same time, atherosclerotic plaques feeding can be considered as a syndrome of Early Vascular Aging (EVA) at a young age⁴.

This syndrome was described recently. It is believed that it is associated with both the influence of the risk factors and can be genetically determined. It is considered that EVA

¹ G. K. Hansson, "Inflammation, atherosclerosis, and coronary artery disease", *N Engl J Med* num 352 (2005): 1685–95.

² P. P. Toth, "Subclinical atherosclerosis: what it is, what it means and what we can do about it", *Intern J Clin Pract* Vol: 62 num 8 (2008): 1246-1254. DOI: 10.1111/j.1742-1241.2008.01804.x.

³ H. Oh; S. C. Wang; A. Prahash; M. Sano; C. S. Moravec; G. E. Taffet; L. H. Michael; K. A. Youker; M. L. Entman & M. D. Schneider, "Telomere attrition and Chk2 activation in human heart failure", *Proceedings of the National Academy of Sciences of the United States of America* Vol: 100 num 9 (2003): 5378–5383.

⁴ J. L. Cavalcante; J. A. Lima; A. Redheuil y M. H. Al-Mallah, "Aortic stiffness: current understanding and future directions", *J Am Coll Cardiol*. Vol: 57 num 14 (2011): 1511—1522.

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is one of the manifestations of General biological aging that develops prematurely. The arteriosclerosis correlates not only with cardiovascular diseases but with the overall mortality⁵.

We have identified morphometric parameters to study the structural changes of arteries in different age groups that correspond to those that are used in clinical practice with the participation of ultrasound methods. Taking into account the fact that it is currently impossible to measure individual layers of intima and media, the use of morphological methods is very relevant.

Thus, the aim of our research was to study the features of morphometric parameters of the arterial wall and their correlation with the individual CVD risk factors.

Methods

The carotid vessels of 106 accident victims (average age $53,3 \pm 20,43$ years) were studied. Previously, all the test subjects were taken to the hospital, the autopsy of the deceased was carried out no later than 24 hours of death. The medical histories of the deceased were studied in order to find out clinical and laboratory data. In this case, the following exclusion criteria were formed: pregnancy, cancer and cardiovascular diseases, any other chronic diseases that could be found out during the study of the anamnesis or the autopsy. Two groups were identified for the study – the younger age group (men ≤ 45 years, women ≤ 55 years, 56 objects) and the older group (50 objects). During the forensic examination of the corpse, an electronic caliper was used to measure the external (D_{ex} , mm) and internal (D_{in} , mm) diameters of blood vessels, as well as the thickness of the subcutaneous fat layer (SAT) on the abdomen (increased development was considered > 2 cm). The studied biochemical parameters: glucose level (hyperglycemia) was found at the level of $\geq 6,1$ mmol / l), urea level (urea level) of blood (2.4 – 8.3 mmol/l was considered normal).

The segments of the carotid vessels were fixed in a 10% solution of formalin, dehydrated, and poured into paraffin. Then, sections were made with a thickness of 3-5 mm and stained with hematoxiline and eosin, as well as a special method for detecting of collagen and elastic fibers – Weiger-van Gieson. Photos were taken from ready-made histological preparations and morphometric studies were performed using AxioVision (CarlZeiss) and ImageJ (NIH USA) programs. The following parameters were identified: carotid artery wall thickness (h , μm), intima thickness (μm), and media thickness (μm). The thickness of the intima-media complex (IMT - intima-media thickness) obtained in μm was converted to mm for comparison with this parameter of clinical practice detected by ultrasound methods. A $\text{IMT} > 0.9$ mm was considered a thickening. The cross-sectional area of the arterial wall was also calculated using the formula $S_{sec} (\text{mm}^2) = \pi \cdot (D_{ex}^2 - D_{in}^2) / 4$. The area of the inner ($S_{D_{in}}$, mm^2) and outer ($S_{D_{ex}}$, mm^2) diameters was found by the formula $S_x = \pi \cdot D_x^2 / 4$. The specific volume of smooth muscle cells was determined using a grid of equidistant points. The tortuosity Factor of elastic membranes (EM) was also calculated: $\varphi = L_{EM} / L$, where L_{EM} is the length of the elastic membrane, and L is the length of the line connecting the beginning and

⁵ L. F. Cherkas; A. Aviv; A. M. Valdes; J. L. Hunkin; J. P. Gardner; G. L. Surdulescu; M. Kimura and T. D. Spector, "The effects of social status on biological aging as measured by white-blood-cell telomere length", *Aging Cell* num 5 (2006): 361-365.

end of the EM. The output of the arteries was estimated by the coefficients of Vogenvort (V) and Kernogan (K) which were calculated using the following formulas: $W=S_{sec}/S_{Din}$; $K=h/r$ - where r (μm) is the radius of the vessel. The statistical processing was performed using the IBM SPSS Statistics V.25 application software package. The data is presented as the sample average (M) and its standard deviation (SD). The distribution in the samples was close to normal. The student's T-test and the Mann-Whitney U-test were used for determine the significance of differences between groups. The Spearman's rank correlation coefficient was used to study the relationships between the most significant morphometric parameters and CVD risk factors. The bond strength was evaluated on a scale from ± 0.7 to ± 1 – strong, from ± 0.3 to ± 0.699 – medium, and from 0 to ± 0.299 -weak. The frequency analysis was performed using the conjugacy tables based on the exact Fischer criterion. The level of statistical significance was considered to be $p < 0.05$.

Results

The clinical characteristics of the groups are shown in table #1. There were 74 male and 32 female corpses of the 106 dead. Since age over 45 for men and over 55 for women is the most important non-modifiable risk factor for CVD, two age groups were identified. There were men who died before 45 years and women are before 55 years they were determined as the younger group ($n=56$, average age 36.57 ± 7.77 years). The remaining objects ($n=50$) were assigned to the older group (71.04 ± 13.45 years). There were no significant sex differences between the groups ($p=0.677$). The increase in laboratory parameters above the threshold values were more often detected in the older age group. The glucose levels greater than 6.1 mmol / l were detected in 34% of cases versus 12% ($p=0.011$). The increased development of subcutaneous fat in the abdomen was detected in 46% of cases against 25% ($p=0.027$). A urea level of more than 8.3 mmol / l was detected in 22% of cases against only 2% ($p=0.044$). There were significant differences between the groups based on these indexes. In the younger age group, glucose levels were 16% lower ($p=0.004$), and urea levels were 28% lower ($p=0.005$). The thickness of the pancreas had no statistically significant differences between the groups ($p=0.162$).

Parameter	General group (n=106)	Younger group (n=56)	Older group (n=50)	
Age, years (M \pm SD)	53.3 \pm 20.43	36.57 \pm 7.77	71.04 \pm 13.45	0.001
Men, % (n)	70 (74)	68 (38)	72 (36)	0.677
Hyperglycemia, %, (n)	22 (24)	12 (7)	34 (17)	0.011
Glucose level, mmol/L, (M \pm SD)	6.12 \pm 1.5	5.63 \pm 1	6.65 \pm 1.76	0.004
SAT>2cm, %, (n)	35 (37)	25 (14)	46 (23)	0.027
SAT, cm, (M \pm SD)	2.14 \pm 1.17	2.02 \pm 1.16	2.29 \pm 1.18	0.162
Urea, mmol/L, (M \pm SD)	6.9 \pm 2.74	5.33 \pm 2.08	7.7 \pm 2.7	0.005
Uraemia, %, (n)	11 (12)	2 (1)	22 (11)	0.044

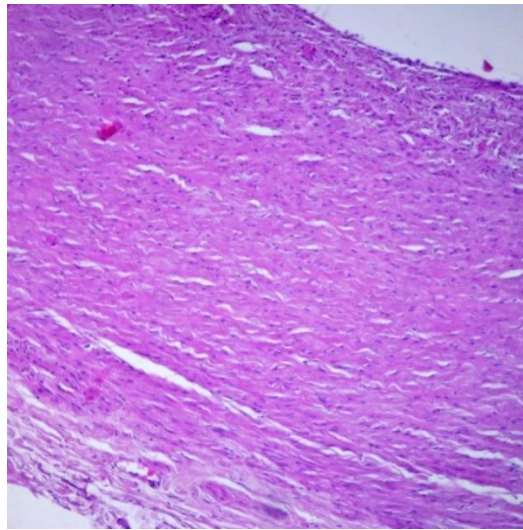
Note. M - mean value, SD - standard deviation, p - level of statistical significance of differences between the younger and older groups. SAT - abdominal subcutaneous adipose tissue.

Table 1
Clinical characteristics of the deceased included in the study

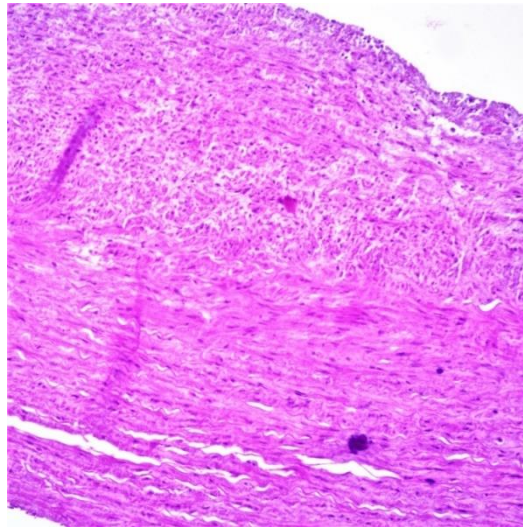
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Morphometric characteristics of carotid vessels are indicated in table #2. In the older group, there was a statistically significant increase in the parameters that were measured during macroscopic examination of the corpse – external and internal diameters, the radius of the arteries. The cross-sectional area of the vascular wall, as well as the area of internal and external diameters, also increased significantly in the older group.

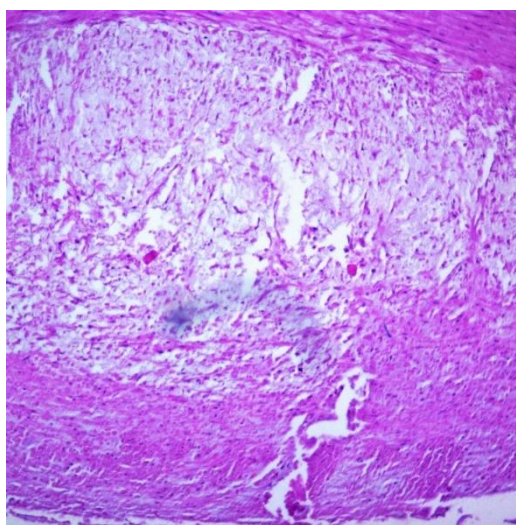
The thickening of the inner and middle layers, which occurs with age, is demonstrated. The thickened (>0.9 m) intima-media complex and atherosclerotic vascular lesion were more common in the older group than in the younger group. It is also worth noting that IMT parameter >0.9 mm was less common in both age groups than atherosclerotic plaques (Fig. 1).



a



b



c

Note. a - variant of the normal structure, b - visually thickened intima, c - atherosclerotic plaque. Magnification X10, hematoxylin-eosin.

Fig. 1

Histological variants of the arterial wall in the younger age group

There was an increase in the coefficient of convolution of the fenestrated elastic membranes and a decrease in the specific volume of smooth myocytes in the middle layer of vessels in the older group. Indexes reflecting impaired circulation in the arteries were higher in the older age group than in the younger.

Parameter	General group (n=106)	Younger group (n=56)	Older group (n=50)	
D _{ex} , mm	7.31±1.05	6.88±1.11	7.73±0.78	0.001
D _{in} , mm	5.6±0.91	5.41±1.1	5.77±0.65	0.007
R, mm	2.79±0.47	2.71±0.58	2.87±0.33	0.013
S _{sec} , mm ²	17.62±6.76	14.16±4.82	20.95±6.73	0.000
S _{Dex} , mm ²	42.92±12.33	38.17±12.78	47.49±10.05	0.000
S _{Din} , mm ²	25.29±8.41	24±10.06	26.53±6.31	0.007
Intima, μm	131.44±70.32	96.01±51.77	165.6±69.26	0.000
Media, μm	656.88±128.22	625.13±124.16	687.49±125.64	0.01
IMT, μm	785.14±161.59	722.07±156.66	845.95±142.93	0.000
IMT>0.9mm, %, (n)	22 (24)	11 (6)	36 (18)	0.005
Atherosclerotic plaques presence, %, (n)	39 (42)	21 (12)	60 (30)	0.000
Atherosclerotic plaques area, %	40.47±16.81	25±9.04	46.66±15.16	0.012
φ	0.79±0.11	0.77±0.1	0.82±0.11	0.027
Specific volume of SMCs	6.48±2.98	8.86±2.26	4.76±2.14	0.000
	0.71±0.29	0.62±0.29	0.8±0.26	0.005
	0.31±0.1	0.27±0.11	0.35±0.09	0.002

Note. M - mean value, SD - standard deviation, p - level of statistical significance of differences between the younger and older groups. D_{ex} - external diameter, D_{in} - inner diameter, R – radius, S_{sec} , mm^2 - the cross-sectional area of the artery wall, S_{Dex} , mm^2 - the area of the arterial wall in the external diameter, S_{Din} , mm^2 - the area of the arterial wall in the inner diameter, IMT - intima-media thickness, ϕ - the Tortuosity Factor of elastic membranes, SMCs - smooth muscle cells, W - Vogenvort index, K - Kernogan index.

Table 2
Characteristic of morphometric parameters of the arterial wall (M±SD)

Younger group					
	Age	Sex	Glucose	SAT	Urea
D_{ex}	r=0.544 p=0.000	r=-0.225 p=0.12	r=0.16 p=0.272	r=-0.095 p=0.515	r=-0.204 p=0.417
D_{in}	r=0.298 p=0.037	r=-0.193 p=0.183	r=-0.13 p=0.93	r=-0.048 p=0.742	r=-0.094 p=0.711
S_{sec}	r=0.328 p=0.000	r=0.052 p=0.721	r=0.353 p=0.013	r=0.299 p=0.037	r=-0.157 p=0.534
Intima	r=0.488 p=0.000	r=-0.158 p=0.255	r=0.51 p=0.000	r=0.62 p=0.000	r=-0.011 p=0.964
Media	r=0.624 p=0.000	r=-0.104 p=0.455	r=0.369 p=0.006	r=0.35 p=0.009	r=0.207 p=0.411
IMT	r=0.651 p=0.000	r=-0.114 p=0.412	r=0.437 p=0.001	r=0.449 p=0.001	r=0.161 p=0.523
Atherosclerotic plaques	r=0.489 p=0.000	r=0.092 p=0.51	r=0.543 p=0.000	r=0.471 p=0.000	r=0.28 p=0.26
ϕ	r=0.242 p=0.143	r=0.122 p=0.467	r=0.035 p=0.836	r=-0.028 p=0.866	r=-0.117 p=0.644
Specific volume of SMCs	r=0.326 p=0.104	r=-0.102 p=0.619	r=0.255 p=0.208	r=-0.112 p=0.585	r=-0.313 p=0.297
W	r=0.297 p=0.063	r=-0.104 p=0.522	r=0.308 p=0.053	r=0.205 p=0.205	r=-0.103 p=0.416
K	r=0.31 p=0.052	r=-0.19 p=0.24	r=0.261 p=0.104	r=0.11 p=0.497	r=0.003 p=0.992
Older group					
	Age	Sex	Glucose	SAT	Urea
D_{ex}	r=0.74 p=0.608	r=0.07 p=0.631	r=0.137 p=0.344	r=-0.126 p=0.354	r=-0.038 p=0.818
D_{in}	r=-0.164 p=0.255	r=0.036 p=0.805	r=-0.154 p=0.284	r=-0.073 p=0.595	r=-0.15 p=0.355
S_{sec}	r=0.323 p=0.022	r=0.122 p=0.398	r=0.35 p=0.013	r=-0.177 p=0.191	r=0.215 p=0.183
Intima	r=0.461 p=0.001	r=0.023 p=0.873	r=0.298 p=0.035	r=-0.018 p=0.895	r=0.331 p=0.037
Media	r=0.311 p=0.028	r=0.014 p=0.924	r=0.329 p=0.02	r=-0.253 p=0.06	r=0.275 p=0.086
IMT	r=0.446 p=0.001	r=-0.002 p=0.992	r=0.427 p=0.002	r=-0.232 p=0.086	r=0.323 p=0.042
Atherosclerotic plaques	r=0.621 p=0.000	r=0.118 p=0.415	r=0.462 p=0.001	r=0.186 p=0.17	r=0.102 p=0.53
ϕ	r=0.521 p=0.001	r=-0.151 p=0.353	r=0.299 p=0.061	r=-0.01 p=0.953	r=0.104 p=0.548
Specific volume	r=-0.422	r=-0.102	r=-0.087	r=0.72	r=0.041

of SMCs	p=0.01	p=0.553	p=0.376	p=0.675	p=0.814
W	r=0.483 p=0.007	r=-0.016 p=0.934	r=0.508 p=0.004	r=-0.086 p=0.65	r=0.297 p=0.117
K	r=0.482 p=0.007	r=-0.059 p=0.757	r=0.542 p=0.002	r=-0.11 p=0.564	r=0.292 p=0.125

Note. p - level of statistical significance. SAT - abdominal subcutaneous adipose tissue, D_{ex} - external diameter, D_{in} - inner diameter, IMT - intima-media thickness, ϕ - the Tortuosity Factor of elastic membranes, SMCs - smooth muscle cells, W - Vogenvort index, K - Kernogan index.

Table 3
The results of Spearman's correlation analysis

A Spearman correlation analysis was performed to assess the strength of the correlation between individual morphometric parameters of carotid arteries and certain CVD risk factors. Its results are shown in table 3. There was a significant correlation between age and area of atherosclerotic lesion, it was more manifested in the older age group. At the same time, the thickness of the inner and middle layers, as well as their thickness in the complex, shown a significant correlation with age, it was more manifested in the younger group. The coefficient of convolution of fenestrated elastic membranes and specific volume of the media smooth myocytes were significantly correlated with age only in the older age group. The remaining risk factors had a significant correlation mainly with markers of atherosclerosis (thickness of the intima, media, IMT and atherosclerotic plaques area).

The blood glucose levels and these markers had a significant correlation in both the younger and older age groups. The thickness of the pancreas significantly correlated with morphometric parameters only in the younger group. The blood urea levels have shown a statistically significant correlation with intima and IMT in the older age group. A statistically significant correlation between sex and morphological parameters of the arterial wall was not established in any of the groups.

Results discussion

The marked frequency and severity of markers of atherosclerosis in the older group may characterize the age-related remodeling of the arteries. However, similar changes in the vascular wall were detected at a young age. Thus, $IMT > 0.9$ mm was observed in 11% of cases, and the presence of atherosclerotic plaques – in 21% of cases in the younger age group. At the same time IMT is considered an unfavorable prognostic marker for the development of cardiovascular diseases. Thus, it can be stated that structural remodeling of the vascular wall can begin at a young age. This is confirmed by the concept of early vascular aging⁶.

It is considered that the greatest danger of risk factors is for young people. Their effect on the vascular wall in older people is less manifested. There is evidence that abdominal obesity and dyslipidemia in older age lose their connection with vascular wall remodeling⁷. The results were similar in our work where the marker of obesity was an increase in the thickness of the abdominal wall more than 2 cm.

⁶ Yu. A. Karpov, "How to prevent early vascular aging in patients with arterial hypertension? Atmosfera", *Novosti kardiologii - Atmosphere. Cardiology news* num 3 (2016): 2-10.

⁷M. A. Druzhilov y T. Y. Kuznetsova, "Visceral obesity as risk factor of early vascular aging", *Cardiologija – Cardiology* Vol: 2 num 56 (2016): 52-6.

The hyperglycemia is considered to be an age-related disease according to the references. An increase of blood glucose level leads to damage to the endothelium, hypertrophy of the SMC and the formation of collagen layers between them. In the end, elasticity decreases, it means that the rigidity of the vascular wall increases⁸.

The increased urea content in the blood leads to the death of endothelial cells and it can lead to the development of oxidative stress in the intima of the vascular wall. As a result, we can see increase of the permeability of the vascular wall, edema of the inner layer and the accumulation of cellular elements in it⁹.

In our study, the level of urea was a risk factor, which has demonstrated a correlation with the older group. In this case, the correlation was established with intima and IMT, which corresponds to the mechanisms which were shown above.

Conclusion

The onset of pathological changes in the vascular wall at a young age is associated with CVD. There is a resistance of the arteries to such a risk factor as obesity with age. The level of glucose in the blood retains its aggressive effect on the blood vessels throughout life. The level of urea is associated with changes in the tunica intima of the arteries in older age. The in-depth knowledge in this area can be useful in the study and prevention of risk factors for heart disease.

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