A&L
ISSN 2709-0205
Vol 3 Issue 1 (2022)
https://doi.org/10.47855/jal9020-2022-1

Age features of functional condition of microvessel endothelia

Ivanna A. Antonyuk-Shcheglova, Olena V. Bondarenko, GeorgijV. Duzhak, Oleg V. Korkushko, Svitlana S. Naskalova, Larysa A. Bodretska, Iryna S. Shapovalenko, Valeri B. Shatilo

D. F. Chebotarev Institute of Gerontology NAMS of Ukraine, Kyiv, Ukraine

https://doi.org/10.47855/jal9020-2022-1-2

Correspondence: elena_bondarenko@ukr.net

Received: 18.02.2021; Accepted: 31.03.2022; Published: 05.04.2022

Abstract. Our research aimed to investigate age-related changes in the functional state of the microvascular endothelium.Матеріали та методи. 390 people were surveyed, divided by age into the following groups: 20-29 (n = 31), 30-39 (n = 22), 40-49 (n = 45), 50-59 (n = 72), 60- 69 (n = 129) and 70-79 years (n = 91). The functional state of the microvascular endothelium was studied by laser Doppler flowmetry (LDF) on the BLF-21D (Transonic Systems Inc, USA) in the middle third of the inner surface of the forearm. The turbidimetric method studied platelet aggregation activity on a two-channel laser platelet aggregation analyzer 23 LA (Biola, Russia). Blood viscosity was determined using a rotary viscometer AKR-2 (Russia) at shear rates of 10-200 s-1 with the calculation of the deformation index (IDE) and erythrocyte aggregation index (IAE). As a ***result*** of the research, it was found that there was a statistically significant decrease in the cutaneous rate of skin blood flow volumetric velocity (SBF) at rest and at the peak of post-occlusive reactive hyperemia, as well as a reduction in the recovery time of the CMT to baseline. indicating endothelial dysfunction with ageing from the age of 50-59 years. In persons older than 60 years there is an increase in endothelial dysfunction, which is accompanied by deterioration of hemostasis: increased blood viscosity, increased both spontaneous and induced platelet aggregation activity. Blood viscosity increases statistically significantly from the age of 40. We have drawn the following ***conclusions****:* the development of endothelial dysfunction with ageing is characterized by changes in the vasomotor function of the endothelium, its antiplatelet, antiadhesive and antithrombotic properties, which leads to the development of vascular pathology in the elderly.

Keywords: functional state of the endothelium; platelet aggregation

Today, the biological age of blood vessels is considered to be one of the most important factors of ageing, and understanding the mechanisms underlying vascular pathophysiological changes in the vascular bed is important and necessary for the development of new methods of pathogenetic treatment.

It is the endothelial dysfunction that occurs with age that underlies vascular ageing and leads to the development of various diseases. It is known that the ageing process is characterized by a decrease in endothelium-dependent vasodilation. In the elderly, the activity of endothelial NO synthase decreases and, as a result, the formation of nitric oxide (NO), which, in turn, leads to platelet activation and increased atherogenesis [1, 2].

In the works of V. Yu. Lyshnevskaya (2004) showed that the level of endothelial relaxing factor and prostacyclin decreases significantly with age. At the same time, there was an increase in vasoconstrictors: endothelin - 1 and thromboxane A2 [3].

The development of endothelial dysfunction with ageing is due to both functional and structural changes in the endothelium. Thus, in experimental studies, O.K. Kulchytsky and co-authors [4] showed that with age in old rats there is a decrease in NO levels in blood plasma and vascular wall, due to insufficient synthesis by endothelial cells. With ageing, the activity of NO synthase (NOS) changes the activity of constitutional NOS decreases and the activity of inducible NOS increases. Decreased NO-synthase activity in blood plasma and vascular walls may be due to a deficiency of the substrate for the synthesis of NO L-arginine. Moreover, it was noted that arginase activity and urea levels decrease with age, indicating a violation of the neoxygenase pathway of conversion of L-arginine as the main NO substrate.

Another reason for the development of endothelial dysfunction is structural changes in the vascular endothelium. Although endotheliocytes are a short-lived, self-renewing population of cells, there is evidence that the restoration of functionally complete endothelium occurs in people only up to 30-35 years of age [5]. This is due to both genetic preconditions and changes in the environment surrounding the endothelium (plasma composition, rheological properties of blood). Of particular note is the study of KG Sarkisov and co-authors [6], which showed that biopsies of the skin of healthy people (biopsies taken in almost the same places where the endothelial function of the microcirculatory tract of the vascular system was studied) are significant ultrastructural changes in endothelial cells. In the elderly there is heterochromia of the nucleus, depletion of ribosomes of the membranes of the granular endoplasmic reticulum, disrupted the structure of mitochondria in the form of swelling, death of cristae, destruction of individual organelles, increasing the number of primary and secondary lysosomes. The structure of microfibrils is also disturbed. Age-related changes in endothelial cells are accompanied by rearrangement of the basal layer of the capillary wall - its thickening due to the formation of multilayered basement membranes and an increase in the number of pericytes.

Age-related changes in the endothelium not only reduce its vasomotor function but also have a negative impact on the antiplatelet, antiadhesive and antithrombotic properties of the endothelium [7,8]. Thus, in response to post-occlusive reactive hyperemia in the elderly, the severity of vascular dilatation decreases and the spastic reaction to adrenaline increases, reduced antithrombotic activity of the endothelium is observed.

In the elderly, platelets' blood viscosity, aggregation, and adhesive activity increase [1]. These age-related changes are even more significant in modelling the impact of stressors on the body. Thus, with the introduction of adrenaline in young people there was a slight increase in blood viscosity, while in people older than 60 years there was a significant increase in blood viscosity at all shear rates, in addition, they showed a statistically significant increase in erythrocyte aggregation activity. At the same time, the processes of blood coagulation intensified in the elderly (plasma recalcification time decreased, plasma tolerance to heparin increased, blood thromboplastin activity increased).

Thus, the study of changes in endothelium-dependent vasodilation with ageing, as well as increasing with age, blood viscosity, aggregation and adhesive activity of platelets may be important in diagnosing the development of vascular pathology in the elderly. Especially given the extremely acute situation in the world regarding the COVID-19 pandemic, in the pathogenesis of which the development of endothelial dysfunction plays a primary role [9].

Materials and methods of research.

The indicators of microcirculation and hemostasis and anthropometric indicators (body mass index, waist circumference) in 390 people of different ages surveyed in the period from 1998 to 2018 were analyzed. The distribution into age groups was as follows: 20-29 years (n = 31), 30-39 (n = 22), 40-49 (n = 45), 50-59 years (n = 72), 60-69 years (n = 129) and 70-79 years (n = 91). The study did not include individuals with exacerbations or decompensated conditions of chronic diseases of the cardiovascular, digestive, respiratory systems, with type I and II diabetes, with cancer.

To assess the state of microcirculation used the technique of laser Doppler flowmetry (LDF), which allows to record changes in blood flow in the microcirculatory channel and monitor the response of microvessels in functional tests used to determine the functional state of vascular endothelium.

The LDF method is based on the measurement of the Doppler component in the spectrum of the reflected laser signal, which is scattered on the formed elements of blood (erythrocytes) in microvessels. The LDF signal quantitatively characterizes blood flow in microvessels (arterioles, capillaries, venules). The volumetric velocity of skin blood flow volumetric velocity (SBF) was determined using a dual-channel laser Doppler flowmeter BLF-21D (Transonic Systems Inc, USA). The functional state of the microvascular endothelium was determined by the method of O. V. Korkushko and V. Yu. Lishnevskaya [10]. At first, the volumetric rate of skin blood flow volumetric velocity at rest was measured. (SBF_{in}). Then a functional test with reactive hyperemia was performed, for the creation of which the vessels of the shoulder were squeezed for 3 min with a cuff in which the pressure exceeded the systolic blood pressure of the subject by 50 mm Hg. After the restoration of blood flow (cessation of compression) there is an increase in blood supply to tissues due to vasodilation due to the release of nitric oxide microvessels by the endothelium. During this period, the maximum volumetric velocity of skin blood flow volumetric velocity was determined (SBF_{max}) and the length of the SBF recovery period to baseline values (trec). The higher both indicators, the better the functional state of the microvascular endothelium.

Platelet aggregation activity was determined on a two-channel laser platelet aggregation analyzer 23 LA (Biola, Russia) by the turbidimetric method. Blood samples for the study were performed in a silicone tube with a 3.8% sodium citrate solution in a volume ratio of 1: 9 (final concentration of citrate in vitro 0.38%). Platelet-rich plasma was used. The spontaneous and induced platelet aggregation level on light transmission curves was evaluated. ADP at a final concentration of 5 μmol / l and adrenaline at a final concentration of 1 μmol / l were used as inducers.

Blood viscosity was determined using a rotary viscometer AKR-2 (Russia) at shear rates of 10-200 s^{-1} with the calculation of the deformation index (IDE) and erythrocyte aggregation index (IAE). IAE was calculated as the ratio of blood viscosity at a shear rate of 20 s^{-1} and blood viscosity at a shear rate of 100 s^{-1}. IDE is the ratio of blood viscosity at a shear rate of 100 s^{-1} and a shear rate of 200 s^{-1}[11].

Data were processed by Statistica 7.0 (StatSoft Inc). Variation statistics for the data are given as the mean ± standard error. The difference was considered significant at $p < 0.05$.

Results and discussion

The indicator of SBF at rest (SBF_{in}) characterizes the general state of perfusion of fabrics at the level of a microcirculatory channel From 50-59 years there is a statistically significant decrease in ОШШШКвих, which becomes pronounced after 60 years (Tab. 1). When conducting a test with the creation of post-occlusal reactive hyperemia, it was shown that there is also a significant decrease in OSHSHK at the peak of post-occlusal reactive hyperemia in this age period.

Table 1

Indicators of SBF at the test with reactive hyperemia in people of different age

Indicator	20-29 years (n = 31)	30-39 years (n = 22)	40-49 years (n = 45)	50-59 years (n = 72)	60-69 years (n = 129)	70-79 years (n = 91)
SBF at rest, ml / (min · 100 g of tissue)	1.29 ± 0.05	1.13 ± 0.07	1.09 ± 0.06	1.03 ± 0.04*	0.97 ± 0.03*	1.02 ± 0.03*
Maximum SBF at the height of reactive hyperemia, ml / (min · 100 g of tissue)	6.74 ± 0.39	6.12 ± 0.37	6.33 ± 0.52	5.87 ± 0.17*	5.65 ± 0.29*	5.8 ± 0.29*

SBF recovery time to initial level, s	140.7 ± 10.0	124.9 ± 12.1	128.3 ± 7.0	104.7 ± 4.4*	105.3 ± 4.2*	104.8 ± 4.9*

Note: *- statistical significance in comparison with the group of 20-29 years: $p < 0.05$

In addition, with age, the duration of the period of restoration of blood flow to the initial level decreases. Taking into account the literature data [12], the presented results indicate a violation of endothelium-dependent vasodilation, most likely due to reduced endothelial synthesis of nitric oxide. Thus, the above data indicate the impaired vasomotor function of the endothelium in people over 50 years, which, in our opinion, is associated with age-related deterioration of endothelial production of vasodilators, including nitric oxide.

With endothelial dysfunction, hemostasis is known to worsen: blood viscosity increases, spontaneous and induced platelet aggregation activity increases. As a result of these changes, the incidence of cardiovascular pathology in the elderly increases [9].

We have shown a statistically significant increase in blood viscosity in groups over 40 years of age at different shear rates (Tab. 2).

Table 2

Blood viscosity, aggregation index and erythrocyte deformity index in people of different ages M ± m

Indicators		**20-29 years (n = 31)**	**30-39 years (n = 22)**	**40-49 years (n = 45)**	**50-59 years (n = 72)**	**60-69 years (n = 129)**	**70-79 years (n = 91)**
Blood viscosity, CPR at speeds rates	200 s^{-1}	3.36 ± 0.07	3.54 ± 0.08	3.63 ± 0.09*	3.78 ± 0.06*	3.79 ± 0.05*	3.79 ± 0.04*
	100 s^{-1}	3.57 ± 0.07	3.74 ± 0.09	3.81 ± 0.09*	3.95 ± 0.06*	3.93 ± 0.06*	3.95 ± 0.04*
	50 s^{-1}	3.78 ± 0.07	3.99 ± 0.01	4.05 ± 0.09*	4.20 ± 0.06*	4.17 ± 0.06*	4.18 ± 0.05*
	20 s^{-1}	4.02 ± 0.08	4.26 ± 0.11	4.3 ± 0.1*	4.47 ± 0.06*	4.45 ± 0.06*	4.46 ± 0.05*
	10 s^{-1}	4.16 ± 0.08	4.39 ± 0.12	4.5 ± 0.1*	4.66 ± 0.07*	4.64 ± 0.07*	4.63 ± 0.05*
IAE, in. units		1.19 ± 0.01	1.19 ± 0.01	1.24 ± 0.03*	1.26 ± 0.01*	1.27 ± 0.05*#	1.27 ± 0.05*#
IДE, in. units		1.16 ± 0.01	1.16 ± 0.01	1.11 ± 0.02*	1.11 ± 0.00*	1.10 ± 0.01*#	1.10 ± 0.01*#

Note: *- statistical significance in comparison with the group of 20-29 years: $p < 0.05$

Age-related changes in blood viscosity with ageing are due to the influence of plasma and cellular factors. Plasma factors in older age groups include an increase in total cholesterol and a decrease in the concentration of total cholesterol TG to HDL-C. Among the cellular factors are age-related changes in the physicochemical properties of erythrocytes. Thus, with age, the aggregation capacity of erythrocytes increases (Tab. 2), as evidenced by the growth of IAE. Another factor that affects the viscosity of the blood is to reduce the deformation of erythrocytes and increase their stiffness.

Changes in the functional state of platelets play an important role in the increase in blood viscosity. Spontaneous platelet aggregation activity increases statistically significantly in the elderly (Tab. 3). Under the action of inducers (adrenaline, ADP) the aggregation capacity of platelets increases in the age group of 40-49 years.

Table 3

Indicators of platelet aggregation in people of different ages

Indicator	20-29 years (n = 31)	30-39 years (n = 22)	40-49 years (n = 45)	50-59 years (n = 72)	60-69 years (n = 129)	70-79 years (n = 91)
Spontaneous aggregation, % OD**	2,73±0,38	3,0±0,59	2,73±0,4	3,4±0,5	4,09±0,4*	4,45±0,5*
Adrenaline-induced aggregation, % OD**	39,5±3,8	49,2±8,1	57,0±9,3	54,51±4,4*	59,77±4,5*	55,9±4,3*
ADP-induced aggregation, % OD**	40,3±4,6	52,1±6,9	60,23±8,8*	65,6 ±6,3*	72,0±6,1*	68,5±6,8*

Note: *- statistical significance in comparison with the group of 20-29 years: $p < 0,05$;
** - OD - optical density

Thus, the obtained results and literature data indicate a violation of the functional state of the endothelium during ageing - the development of endothelial dysfunction, which not only changes the vasomotor function of the endothelium but also adversely affects its antiplatelet, antiadhesive and antithrombotic properties. All this not only changes vascular reactivity but can also cause the development of vascular pathology in the elderly.

Conclusions:

1. Impaired vasomotor function of the endothelium begins at age 50 and is due to the insufficient formation of nitric oxide by endothelial cells.
2. Endothelial dysfunction leads to impaired microcirculation, increased platelet aggregation, increased blood viscosity, which increases the risk of thrombotic complications in old age.

Author Contributions: All authors participated equally in writing this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Korkushko, O. V.; Duzhak, G. V. Age-related changes in the rheological properties of blood and the state of endothelial function of the microcirculatory vascular bed. *Problems of ageing and longevity* **2011**, 20, 1, 35—52. (in Russian)
2. Babik, B.; Peták, F.; Agócs, S.; Blaskovics, I.; Alács, E.; Bodó, K. & Südy, R. Diabetes mellitus: endothelial dysfunction and changes in hemostasis. *Orvosi hetilap* **2018**, 159, 33, 1335–1345. https://doi.org/10.1556/650.2018.31130
3. Lishnevskaya, V. Y. *The role of destabilization of hemovascular homeostasis in the development of myocardial ischemia in elderly patients with ischemic heart disease*. Abstract. dis. DSC (Medicine): Kyiv, **2004**, p 47 (in Russian)
4. Kulchitsky, O. K.; Nizhankovskaya, O. V.; Potapenko, R. I. et al. Peculiarities of NO-synthase and arginase pathways of L-arginine transformation in the vascular wall of rats of different ages. *Circulation and hemostasis* **2006**, 1, 77-80. (in Russian)
5. Baggio, G. (1999). Occurrence of chronic diseases and their impact on physical disability over the whole spectrum of ageing: from 65 to over 100 years of age. *Zeitschrift fur Gerontologie und Geriatrie* **1999**, 32, 6, 420–424. https://doi.org/10.1007/s003910050139
6. Sarkisov, K. G.; Korkushko, O. V.; Stupina, A. S. et al. Microcirculation and hemorheology in human ageing. *Problems of ageing and longevity* **1998**, 7, 3, 269–278. (in Russian)
7. Seals, D. R.; Jablonski, K. L. & Donato, A. J. Aging and vascular endothelial function in humans. *Clin Sci (Lond)* **2011,** 120, 9, 357–375. https://doi.org/10.1042/CS20100476
8. Wang, M.; Hao, H.; Leeper, N. J.; Zhu, L. & Early Career Committee. Thrombotic Regulation From the Endothelial Cell Perspectives. *Arteriosclerosis, thrombosis, and vascular biology* **2018**, 38, 6, e90–e95. https://doi.org/10.1161/ATVBAHA.118.310367
9. Oliveira, M. R.; Back, G. D.; da Luz Goulart; C., Domingos, B. C.; Arena, R. & Borghi-Silva, A. Endothelial function provides early prognostic information in patients with COVID-19: A cohort study. *Respiratory medicine* **2021**, 185, 106469. https://doi.org/10.1016/j.rmed.2021.106469
10. Korkushko, O.V.; Lishnevskaya, V. Yu; Duzhak, G. V. Age features of the functional state of the microvascular endothelium. Circulation and haemostasis **2007**, 4, 5-11. (in Russian)
11. Berkovsky, A.L *A manual on the study of adhesiveaggregation activity of platelets*. Berkovsky, A.L:Vasiliev, S.A.; Zherdeva, L.V., Eds. Educational manual: Moskow, **2002**, p 62 (in Russian)
12. Berenyiova, A., Drobna, M., Cebova, M., Kristek, F., & Cacanyiova, S. Changes in the vasoactive effects of nitric oxide, hydrogen sulfide and the structure of the rat thoracic aorta: the role of age and essential hypertension. *J Physiol Pharmacol.* **2018**, 69, 4, 1-12. https://doi.org/10.26402/jpp.2018.4.05