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Morphological and functional features of different types of arteriovenous anastomoses in rabbits

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The effectiveness of programmed haemodialysis in patients with chronic kidney disease depends on the formation of permanent vascular access. The morphofunctional characteristics of arteriovenous anastomoses were studied in rabbits, where in the first group the "end to the side" was between the common carotid artery and the external jugular vein, and in the second group the "end to the side" was made using jugular vein tributaries, where the "side" of the anastomosis was formed by the common carotid artery and the "end" by the maxillary and glossofacial veins. Morphological and functional features of the vessels were studied using ultrasound and histological methods. Ultrasound examination of vessels was performed after 2 h and on the 30th day after anastomosis formation ("Philips Lumify", USA, with a linear transducer of 4-12 MHz). Animals were withdrawn from the experiment on the 30th day of observation. It was found that after one month, the diameter of the common carotid artery exceeded the value in intact animals (control group) by 44% (only in rabbits of the 2nd group), the external jugular vein by 2.4 and 2.6 times in animals of the 1st and 2nd groups, respectively. Blood flow velocity in the external jugular vein in the early postoperative period increased by 4.7 and 5.1 times, on the 30th day, by 7.2 and 7.9 times, in animals of the 1st and 2nd groups, respectively. According to the results of light microscopy, in animals of 1st group, the middle layer of the vascular wall is represented by disorganised bundles of smooth myocytes, and areas of their hyperplasia are noticeable. In the venous wall, stasis in the blood vessels of its middle layer, lymphocytic infiltration, and islands of adipocyte accumulation are detected. In animals of the 2nd group, the inner, middle, and outer membranes are identified in the anastomosis area, and endothelial cell nuclei are visualised along the perimeter of the lumen. Smooth myocytes of the middle layer are surrounded by a network of collagen and elastic fibres. In the vein wall, the middle membrane is thickened, smooth myocytes and adipocytes of different calibres are visualised. Thus, the arteriovenous anastomosis end-to-side leads to a significant increase in blood flow and vascular dilatation, and the use of venous tributaries in the formation of vascular access contributes to more effective structural restructuring and fistula functionality.
Key words: arteriovenous fistula; configuration of vascular anastomoses; structural features; functional changes; haemodynamics.

INTRODUCTION

Chronic kidney disease remains a priority medical problem in Ukraine, as it affects the quality of patient's life. The updated KDIGO 2024 guidelines emphasise on the increasing prevalence of the disease worldwide, which ranges from 11 to 15% (over 843 million people) [1]. The same trend is observed in Ukraine, which requires strengthening of preventive measures, improving access to treatment and improving its quality. An important component of replacement therapy in patients with stage 5 chronic kidney disease is programme haemodialysis [2]. The choice of vascular access, in particular, the type of arteriovenous fistula (AVF), plays a significant role in the effective performance of the procedure. The structure and functionality of the formed anastomosis determine the stability of blood flow, the duration of fistula work, and the risk of complications, including its stenosis or thrombosis [3]. Optimisation of the anastomosis formation technique can help to reduce complications and improve the long-term prognosis for patients undergoing programme haemodialysis.

The advantages of AVF over other types of access (arteriovenous prostheses, central venous catheters) are long-term functioning, low risk of infection, less probability of thrombosis [4]. Thanks to the use of the patient's own vessels, the fistula can function for many years with proper care. This reduces the need for repeated surgical interventions and minimises the risk of complications associated with vascular access replacement. AVF is less susceptible to infectious complications compared to synthetic prostheses or catheters, which can become a source of infection. Rapid and constant blood flow through the anastomosis reduces the risk of blood clots formation, which provides reliable access for haemodialysis and improves the patient's quality of life [5].

However, during the formation of AVF, it is not always possible to ensure sufficient blood flow in the vascular bed, and thus prevent or reduce the risks of complications in the early and late postoperative periods [6]. Despite the gold standard, which is the end-to-side anastomosis, the implementation of its various modifications remains relevant [7]. One of these anastomoses is the end-to-side with the use of venous tributaries, which allows to provide the long-term and high-quality functioning of the vascular access. Therefore, it is relevant to study the structural and functional differences of these vascular accesses, which will allow predicting the effectiveness of vascular wall maturation and increase of blood flow velocity. Such results will promote to ensure long-term functionality of the vascular access in patients with chronic kidney disease and improve treatment outcomes, and the quality of haemodialysis.

The aim of the study: to study the structural and functional features of different types of arteriovenous anastomoses in the experiment.

METHODS

Investigation was performed on male Chinchilla rabbits weighing 2.5-3.0 kg. The animals were kept on a standard diet and drinking regime of the vivarium. Studies on animals were conducted in accordance with the existing legal standards (decision of the Ethics Committee of Ivano-Frankivsk National Medical University, protocol No. 130/22 from 22.11.2022). Animals underwent end-to-side arteriovenous anastomoses (1st research group, n = 6) and end-to-side anastomoses using venous tributaries (2nd research group, n = 6) between the common carotid artery (CCA) and external jugular vein (EJV). Access to the vessels was performed by a longitudinal incision (4 cm) in their projection to the right, and the CCA and EJV with its tributaries (maxillary and linguofacial veins) were dissected. The next stage was to form an anastomosis between the CCA and the EJV of the end-to-side type, where the end is the EJV, and the side – CCA (Fig. 1A). When forming an end-to-side anastomosis using venous tributaries the end is the EJV, the side – the incision between the venous tributaries of the EJV with pre-rounded edges of the inlet (Fig. 1B). AVF formation was performed under intramuscular anaesthesia with sodium thiopental (20 mg/kg, "Arterium", Ukraine) and xylazine (sedazine, 10 mg/kg, "Biowet Pulawy Sp. z o.o", Poland). Infiltration anaesthesia (2% lidocaine solution, "Darnytsa", Ukraine) was performed in the incision area. Intraoperatively, animals were administered intravenous heparin (1000 U). To prevent the development of postoperative infectious complications, the animals were administered cefazolin (50 mg/kg, "Arterium", Ukraine). In order to prevent thrombosis, the animals received the platelet P2Y12 receptor inhibitor clopidogrel (Plavix, "SANOFI", France, 20 mg daily, per os).

Morphological and functional features of the vessels were studied using ultrasound and histological methods. Ultrasound examination of the CCA and EJV was performed using a portable Philips Lumify device ("Philips", USA) with a linear transducer (4-12 MHz) 2 h after anastomosis formation and on the 30th day after surgery. During the manipulation, the animals were under thiopental anaesthesia in the position on the back. Blood flow velocity

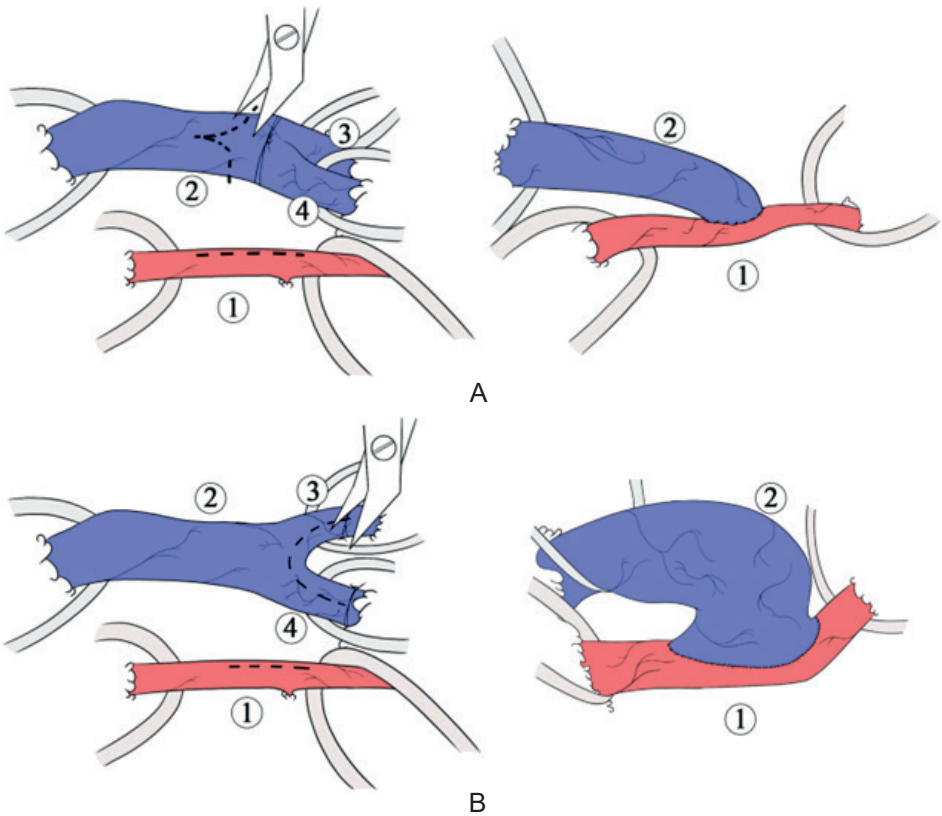

Fig. 1. Technique of vascular access formation end-to-side (A) and end-to-side with the use of venous tributaries (B). 1 – common carotid artery, 2 – external jugular vein, 3 – maxillary vein, 4 – linguofacial vein

(B-Mode regime, Color Doppler), diameter of the newly created vascular access and intact contralateral vessels were measured to assess possible compensatory changes. All studies were performed under standard conditions using appropriate device settings parameters according to the size of rabbit vessels. For comparison, similar studies were performed in intact animals (control group, n = 6).

Animals were withdrawn from the experiment on the 30th day after surgery by an overdose of sodium thiopental. After preparation of the AVF area, the vessels were perfused with saline. The extracted samples were fixed in 10% formalin solution with subsequent paraffin embedding. Transverse sections of paraffin blocks were stained with haematoxylin and eosin. The structural organisation of AVF was studied by light microscopy method. Photographic documentation of micropreparations was performed using a MICROS MC300 (XT) microscope (Austria) with the use of ToupCam 5.1M UHCCD C-Mount Sony digital camera, Adapter AMA075 in ToupTek ToupView software (V3.7.1398).

Statistical data processing was performed using the Excel computer program of the Microsoft Office 365 ProPlus package. Student's t test was used to assess the reliable difference of results in the two compared samples. The difference at $P < 0.05$ was considered statistically reliable.

RESULTS AND DISCUSSION

As a result of the study, the significant changes of vessels diameter and blood flow velocity in the EJV of experimental animals were observed in the early postoperative period and on the 30th day after surgery (Table). After 2 h after the formation of anastomosis, a tendency to increase

the diameters of the CCA and EJV was observed and a reliable increase of blood flow velocity in the EJV in animals of the 1st and 2nd research groups by 4.7 and 5.1 times compared with the baseline values was found. On the 30th day after AVF formation in animals of the 1st group, the diameter of the EJV increased by 2.4 times ($P < 0.05$), and the blood flow velocity increased by 7.2 times ($P < 0.001$) compared with the control. In the 2nd group, where the venous tributaries of the EJV were used to form the anastomosis, the changes of the studied parameters were more significant. In particular, the diameter of the EJV increased by 2.6 times ($P < 0.05$), and the blood flow velocity in it – by 7.9 times ($P < 0.001$). It can be argued that the use of EJV tributaries in the formation of AVF provides better adaptation of the vascular bed to the altered haemodynamics.

In addition, in the 2nd group, a more significant dilation of the CCA (by 43.8%, $P < 0.05$) was also observed, whereas the diameter of the vessel in rabbits of the 1st group did not reliable differ from the control. It is likely that during using venous tributaries, the fistula creates more favourable conditions for the distribution of blood flow, which reduces the load on the arterial wall and promotes its remodelling. A comparative analysis of the parameters revealed a tendency to increase the diameter of the CCA and blood flow velocity in the EJV in animals of the 2nd group compared with the values in rabbits of the 1st. In general, these results reflect the advantages of the modified method of anastomosis formation with the involvement of venous tributaries.

As a result of morphological analysis on the 30th day of the experiment in animals of the 1st group, the anastomosis site does not have a clear wall stratification (Fig. 2). There are loci of endothelial desquamation and fragmentation of the internal elastic membrane. In some fields of view, the middle membrane is represented by disorganised bundles of smooth myocytes, and in others, areas of their hyperplasia are visible.

In the venous wall, stasis in the blood vessels of its middle membrane, lymphocytic infiltration and islands of adipocyte accumulation are detected (Fig. 3).

In animals of the 2nd group, the inner, middle and outer membranes are identified in the anastomosis area (Fig. 4). Endothelial cell nuclei are clearly visualised along the perimeter of the lumen. Smooth myocytes of the middle membrane are surrounded by a network of collagen and elastic fibres. The adventitia is represented by loose connective tissue.

In the vein wall, the middle membrane

Ultrasound parameters of intact vessels, arteriovenous anastomoses end-to-side and end-to-side using venous tributaries after 2 h and on the 30th day after their formation (M ± m, n = 6)

Groups of animals	Diameter CCA, mm	Diameter EJV, mm	Blood flow velocity in EJV, cm/s
Control (intact animals)	1.28 ± 0.14	3.08 ± 0.86	10.34 ± 1.31
Animals underwent end-to-side type of anastomoses formation between CCA and EJV			
<2 h	1.35 ± 0.23	4.54 ± 1.14	48.36 ± 5.17***
30th day	1.52 ± 0.35	7.53 ± 1.31*	74.26 ± 9.46***
Animals underwent anastomoses formation between CCA and EJV end-to-side type using venous tributaries			
<2 h	1.36 ± 0.26	5.64 ± 1.24	52.44 ± 8.32**
30th day	1.84 ± 0.17*	8.12 ± 1.36*	81,18 ± 6.42***

*P < 0.05, **P < 0.01, ***P < 0.001, relative to the values in animals of control group.

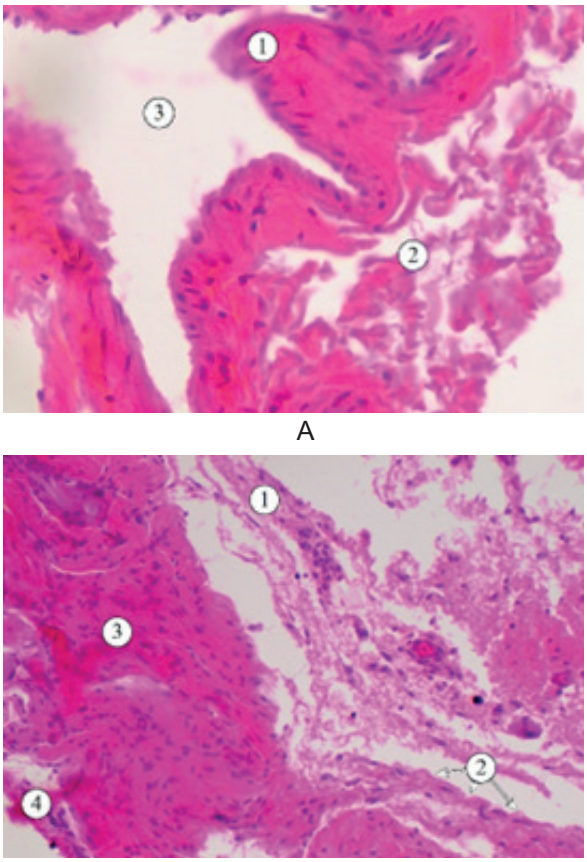

Fig. 2. Structure of the vascular wall (anastomosis site) in animals undergoing end-to-side arteriovenous anastomosis on 30th day of the experiment. Staining: haematoxylin and eosin, A 400×, B 200×. On A: 1 – intima, 2 – media, 3 – lumen; on B: 1 – desquamated endothelium, 2 – fragmented internal elastic membrane, 3 – media, 4 – adventitia

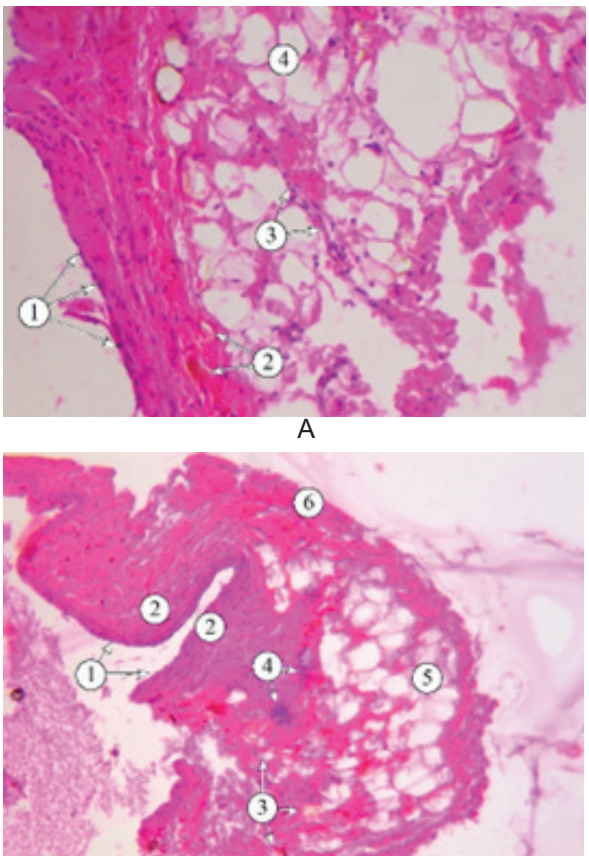

Fig. 3. Structure of the vascular wall (vein site) in animals undergoing end-to-side arteriovenous anastomosis on 30th day of the experiment. Staining: haematoxylin and eosin, A 200×, B 100×. On A: 1 – endothelial cell nuclei, 2 – stasis in the blood vessels of the media, 3 – arteriole in the media, 4 – adipocytes; on B: 1 – endothelial cell nuclei, 2 – intima, 3 – blood vessels of the media, 4 – lymphocytic infiltration of the media, 5 – adipocytes, 6 – adventitia

is thickened (Fig. 5). Smooth myocytes and adipocytes of different calibres are clearly visualised, which cause optical translucency of the wall. Recanalization phenomena are detected.

In general, the histological analysis of arteriovenous anastomoses removed on the 30th day of the postoperative period reflects the changes in the structure of the vascular wall, depending on the type of formed anastomosis. Thus, in animals undergoing end-to-side AVF, zones of endothelial dysfunction with focal areas of endothelial desquamation and moderate infiltration of the vein wall with lymphocytes were observed, which may indicate the development of neointimal hyperplasia and initial manifestations of vascular wall remodelling [8, 9]. In animals with an end-to-side anastomosis using venous tributaries, the formation of all vessel walls, thickening of the veins wall were observed. The structure of the endothelium remained more intact, which may be due to a decrease of mechanical stress and a relatively more even distribution of haemodynamic load [10]. The peculiarities of the structural organisation of veins in animals of this group may reflect better adaptation of vessels to changed blood flow conditions.

Attract attention numerous adipocytes on micropreparations, which may be the result of haemodynamic stress, lipid metabolism disorders, the effects of hypoxia and reoxygenation, as well as an inflammatory reaction [11]. Naturally, after the formation of anastomosis, the nature of blood flow changes dramatically, as a high-speed turbulent flow develops, affecting both the arterial and venous walls. Such changes can lead to the development of endothelial dysfunction and change the mechanical load on the smooth muscle cells of the middle membrane, stimulate their modification, and increase the permeability of the vascular wall [12]. It is worth noting that in case of endothelial dysfunction the

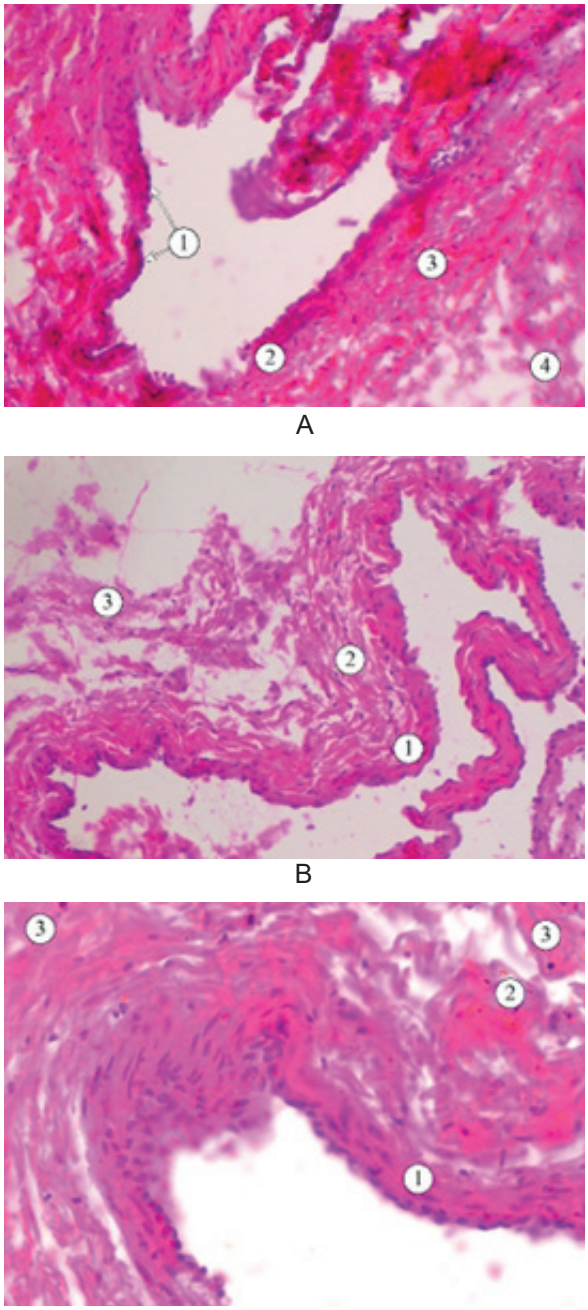

Fig. 4. Structure of the vascular wall (anastomosis site) in animals undergoing end-to-side arteriovenous anastomosis using jugular vein tributaries on 30th day of the experiment. Staining: haematoxylin and eosin, A, B 200×, C 400×. On A: 1 – endothelial cell nuclei, 2 – intima, 3 – media, 4 – adventitia; on B, C: 1 – intima, 2 – media, 3 – adventitia

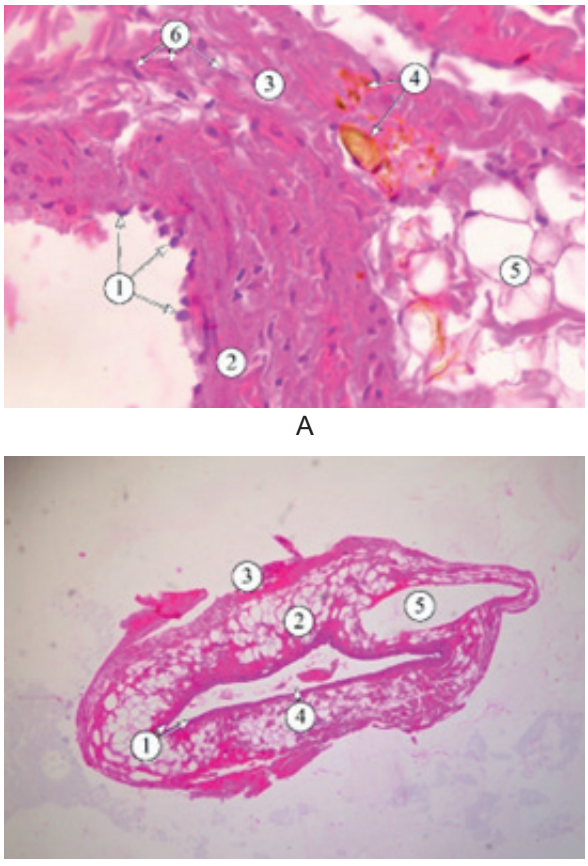

Fig. 5. Structure of the vascular wall (vein site) in animals undergoing end-to-side arteriovenous anastomosis using jugular vein tributaries on 30th day of the experiment. Staining: haematoxylin and eosin, A 400×, B 40×. On A: 1 – nuclei of endothelial cells, 2 – intima, 3 – media, 4 – stasis in the blood vessels of media, 5 – adipocytes in the media, 6 – nuclei of smooth myocytes in the media; on B: 1 – intima, 2 – media, 3 – adventitia, 4 – lumen, 5 – recanalization

regulation of permeability to lipoproteins, especially low-density lipoprotein cholesterol (LDL) is disrupted. Therefore, lipids can penetrate the media and accumulate there. Smooth muscle cells have the ability to active phagocytose of these lipids, turning into foam cells, which forms fatty inclusions [13]. It is worth emphasising on the role of hypoxia and reoxygenation in this process, especially in veins, due to atypical change of pressure and oxygen tension, which activates HIF-1α, stimulates angiogenesis and tissue remodelling, and significantly affects lipid metabolism [14]. There is no doubt about the development of inflammatory process, despite the parenteral perioperative antibiotic prophylaxis. At the same time, macrophages infiltrating the vessel wall can actively accumulate lipids, contributing to the development of atherosclerotic changes even in animals without proatherogenic tendency. Eventually, excessive development of adipocytes can cause stress due to surgical trauma and haemodynamic disorders [15].

It is worth noting that the process of fistula "maturation" usually takes about 4-6 weeks, during which the vessels adapt to increased blood flow and increased pressure [16]. Ultrasound examination allows assessing the functional suitability of vessels and identifying the possible risks and complications at early stages. A thorough clinical examination allows timely detection of anastomotic insufficiency and related disorders, which makes it possible to perform reconstructive interventions in a timely manner to restore AVF functionality and improve the patient's quality of life [17].

Thus, the structural features of the vessels justify the changes of haemodynamic parameters in experimental animals, which reflects the potential advantage of the end-to-end technique using venous tributaries due to a reduced risk of neointimal hyperplasia development, preservation of the morphological organisation of the endothelium, and more favourable adaptation of the vascular wall to altered blood flow conditions.

CONCLUSIONS

The type of arteriovenous anastomoses affects the structural changes of the vessels that form them, as well as haemodynamic parameters. Morphological and functional features of AVF depend on the period of its maturation. The formation of an arteriovenous anastomosis using the end-to-side technique leads to a significant increase of haemodynamics and vascular dilatation, and the use of venous tributaries leads to a more even distribution of blood flow, lower risks of neointimal hyperplasia development, and preserves the morphology of the vessel wall to a greater extent, which reduces the risks of thrombosis and stenosis.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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МОРФОФУНКЦІОНАЛЬНІ ОСОБЛИВОСТІ РІЗНИХ ТИПІВ АРТЕРІОВЕНОЗНИХ АНАСТОМОЗІВ У КРОЛІВ

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Ефективність проведення програмного гемодіалізу пацієнтам із хронічною хворобою нирок залежить від формування постійного судинного доступу. На кролях досліджували морфофункціональні особливості артеріовенозних анастомозів «кінець у бік» між загальною сонною артерією і зовнішньою яремною веною (1-ша група) та «кінець у бік із застосуванням притоків яремної вени», де «бік» анастомозу утворений загальною сонною артерією, а «кінець» – верхньощелепною і язиково-лицевою венами (2-га група). Морфофункціональні особливості судин вивчали за допомогою ультразвукового і гістологічного методів. Ультразвукове дослідження судин проводили через 2-і год і на 30-ту добу після формування анастомозу ("Philips Lumify," США з лінійним датчиком 4–12 МГц). Тварин виводили з експерименту на 30-ту добу спостереження. Встановлено, що через місяць діаметр загальної сонної артерії перевищив значення у інтактних тварин

(контрольна група) на 44% (тільки у кролів 2-ї групи), зовнішньої яремної вени – у 2,4 і 2,6 раза у тварин 1-ї і 2-ї груп відповідно. Швидкість кровотоку у зовнішній яремній вені у ранній післяопераційний період зросла у 4,7 та 5,1 раза, на 30-ту добу – у 7,2 і 7,9 раза відповідно. За результатами світлової мікроскопії у тварин 1-ї групи середня оболонка судинної стінки представлена дезорганізованими пучками гладких міоцитів, помітні ділянки їх гіперплазії. У венозній стінці виявляється стаз у кровоносних судинах її середньої оболонки, лімфоцитарна інфільтрація та острівці скупчення адипоцитів. У тварин 2-ї групи у ділянці анастомозу ідентифікується внутрішня, середня та зовнішня оболонки, по периметру просвіту чітко візуалізуються ядра ендотеліоцитів. Гладкі міоцити середньої оболонки оточені сіткою колагенових та еластичних волокон. У стінці вени середня оболонка потовщена, добре візуалізуються гладкі міоцити та різнокаліберні адипоцити. Таким чином, артеріовенозний анастомоз «кінець у бік» призводить до значного підвищення кровотоку та дилатації судин, а використання венозних притоків при формуванні судинного доступу сприяє більш ефективній структурній перебудові і функціональності фістули.

Ключові слова: артеріовенозна фістула; конфігурація судинних анастомозів; структурні особливості; функціональні зміни; гемодинаміка.

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