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MORPHOLOGICAL ALTERATIONS OF THE CEPHALIC VEIN WALL AS A PAT OF A NATIVE ARTERIO-VENOUS FISTULA FOR CHRONIC HEMODIALYSIS

N. Stoinov, G. Marinov, N. Garbatova, V. Knyazhev[1]

Department of Anatomy, Histology and Embryology and [1]Clinic of Vascular Surgery, Medical University of Varna

ABSTRACT

The creation of an internal arterio-venous fistula according to Brescia and Cimino (1966) changes the functional conditions of cephalic vein and radial artery. Arterial blood enters under high pressure the cephalic vein and turbulent blood flow appears. The pathomorphology of the cephalic vein incorporated in the internal native arterio-venous fistula (NAVF) for chronic hemodialysis was studied. Single portions of the vein were surgically removed from 16 patients aged from 20 to 60 years with failed NAVF and then excised because of repeated NAVF creation (group one) and from 3 patients where the vein was removed during the primary NAVF creation (group two). Light and transmission electron microscopy was used. In the first group, the intima and media of the cephalic vein was much ticker than that of the veins of the patients of the second and control group. The increased thickness of the venous intima was accompanied by an augmented number of smooth muscles cells and appearance of new layers while that of the media was followed by structural changes of its elastic network. These alterations depend on the duration of NAVF functioning.

Key words: permanent vascular access, arterio-venous fistula for hemodialysis, cephalic vein arterialization, intimal thickening

INTRODUCTION

In 1966, Brescia *et al.* (1) reported the first surgical construction of internal arterio-venous fistula by connecting cephalic vein and radial artery. This fistula still remains the best choice of vascular access for chronic hemodialysis among the different types of NAVF (2,3,8,13,15). The created internal arterio-venous Brescia-Cimino's fistula (1) changes the conditions of functioning of the cephalic vein and radial artery. Arterial blood enters under high pressure the cephalic vein and turbulent blood flow appears, which is a probable reason for the increase of "shear stress stimulation" of local growth factors or of their release from platelets and white blood cells (7,12). Progressive neointimal hyperplasia of the venous outflow part of the system and alterations in the venous media is commonly described as "arterialization of the venous wall" (6,7,12). Thickening of cephalic vein intima is considered a basic reason for stenoses of the vein developing at different distance from the fistula and resulting later on in thrombosis, obliteration and NAVF compromizing (7,14). The repeated NAVF creation in such patients remains a basic problem to be solved by different methods depending on the situation and morphological alterations of the cephalic vein and radial artery(1,2).

The aim of the present investigation is to study the morphological changes in the wall of cephalic vein included in the internal Brescia-Cimino's NAVF for chronic hemodialysis. We believe that clarifying the causes could help avoiding the fistula failure. Besides they limit to a great extent the repeated creation of the internal NAVF using the same method.

MATERIAL AND METHODS

Portions of the cephalic vein were surgically removed from two groups of patients. The material of the first group was obtained from 16 patients (13 males and 3 females) aged from 20 to 60 years. In these patients the failed NAVF was excised because of repeated NAVF creation. The material of the second group consisted of cephalic vein surgically removed during the primary NAVF creation (Table 1). As a control group, the segments of the cephalic vein brachial portions without macroscopically visible injuries from 4 cadavers (men aged between 27 and 72 years) were used. The study was carried out after the ethical requirements established in the Medical University of Varna.

Address for correspondence:
G. Marinov, Dept. of Anatomy, Histology and Embryology,
Medical *University, 55 Marin Drinov St, BG-9002 Varna, BULGARIA*
E-mail: anatomia@asclep.muvar.acad.bg

Table 1. Thckness of venous wall

Lifetime of NAVF	n		Age (years)	Individual/group minimal-maximal and average thickness of the wall in μm					
				intima + media		intima		media	
Min.-max. in the group	men	women	Min.-max. in the group	Min.-max. in the group	Average for the group	Min.-max. in the group	Average for the group	Min.-max. in the group	Average for the group
First group									
1 -10 days		2	39-57	177-253	215	15-46	31	162-208	185
1 - 2 years	4		20-53	165-754	533	18 - 257	126	147-605	409
3 - 4 years	3	1	39-56	384-733	537	126-207	167	177-578	370
5 - 8 years	5		25-60	606-832	747	120-517	328	289-680	419
17 years	1		48	495*	495*	316	316	179	179
Second group									
0 days	3		29-50	109-375	210	5-20	10	104-355	200
Control group									
Without NAVF	4		27-72	186-315	263	40-60	45	146-275	218

**Many calcificates and necrotic degradation in the almost entirely obliterating venous-lumen neointima were observed and that was why this vein was not included in the measurement. Strongly broadened vasa vasorum were seen in the whole media and intima close to calcificates.*

Macroscopic observations and specimen harvesting

The removed veins were gently rinsed in saline, carefully investigated by stereomicroscope and fixed as described below. Parts from the cephalic vein, the radial artery and the proper AV anastomosis of the excised fistulas were carefully selected. Representative samples from the cephalic vein only were divided in parts for light and transmission electron microscopy (TEM) examinations.

Light microscopy

The specimens were fixed in neutral-buffered formalin and, after routine processing, they were embedded in paraffin wax. Five-mm sections were cut and one series of them were stained with haematoxylin-eosin, orcein, AZAN, and by the methods of Mallory and Van-Gieson for light microscopy using "Zetopan-Reichert" microscope.

TEM

Tissue specimens were fixed in 3% or 4% glutaraldehyde in phosphate buffer, pH 7.4, postfixed for 2 hours in 1% OsO_4, dehydrated in graded series of ethanol's and embedded in Durcupan. Ultrafine sections were viewed in a JEM 7A and Opton transmission electron microscopes.

RESULTS AND DISCUSSION

Light microscopy

In all patients from the first group, in whom the NAVF has functioned for more than one year, the cephalic vein intima and media both are much ticker than those of the veins of the patients of the second and control groups (Table 1). The average thickness of the intima in the fistulas that have functioned 1-4 years long increases by 3,5-4 times while that in the fistulas that have functioned 5 and more years increases approximately by 7 times than that of the control group.

Fig. 1. Transversal histological section from the wall of the cephalic vein from NAVF after 3-year functioning. Thickened intima with external longitudinal and internal circular SMC layer. Thickened intima with condensed SMC arrangement. HE staining. Magn. 10 x 32.

Smooth muscles in the venous intima of the control group are relatively small in quantity forming a layer of longitudinal bundles divided by a substantial number of rough longitudinal collagen fibres. Increased thickness of the vein intima after its inclusion in the NAVF is accompanied by the greater number of smooth muscles cells (SMC) and the appearance of new layers (Fig. 1).

A 10-30 μm thick circular muscular layer situated inwards to the longitudinal one is formed after one year of functioning in those parts of the wall which intima of approximately 100 μm thick. The circular layer of the intima is thickened substantially reaching up to 300 μm in the veins with longer fistula functioning. It appears as a clearlier expressed intimal layer in some veins. The smooth muscles in the longitudinal intimal layer preserve a typical arrangement in bundles and condense. This layer of the wall in a 60-years old male with 8-years of fistula functioning is intensively developed and its thickness is the same or even bigger than that of the media. In addition, the high density of the smooth muscles resembling an arterial wall is observed. SMC in the circular intimal layer do not form any bundles and are diffusely spread. They are of various density and not quite precise orientation. Stripes of circularly and longitudinally oriented cells appear at times in the layer in case of higher thickness. Collagen fibres in this layer are significant in number, but relatively thinner.

The elastic network of the intima shows a variable structure. An intensively developed, subendothelially located inner elastic membrane is observed in cases with vaguely thickened intima. The elastic membrane in samples with two-layered structure is between the two layers. The elastic fibres in the longitudinal layer are longitudinally oriented and situated on the surface of the muscle bundles. Quite a high density of circular fibres arranged in layers appears in the circular intimal layer building membranes at certain places with the innermost ones forming a subendothelially placed membrane. Some diversions from the above-described scheme may appear, e.g., a thick elastic membrane under the intima with thin longitudinal fibres in it is observed with a 60-years old individual (arterialized intima).

The average media thickness in the fistulas having functioned more than one year is twice bigger than that of the control groups and the veins of uremic patients with newly-formed or short-term functioned NAVF (Table 1). The increased media thickness runs in parallel with the thickening of the smooth muscles bundles in it. It usually preserves an arrangement typical of the vein in the form of circularly oriented bundles being thinner in the inner half of the media. These bundles are of bigger density than the controls while those in the outer half thicken intensively forming at places a dense complete layer resembling the structure of an arterial media. In two cases there are sections with typical arterialization of the vein media expressed as tightly arranged SMC with no connective tissue spaces among them. The increased media thickness is accompanied by structural changes of its elastic network. It is formed by longitudinal elastic fibres in the regions with thickened media but preserved typical structure of the venous wall. They are located on the surface of the muscle bundles and their density and thickness are higher for the relative age. There is a substantial thickening and condensation in the adventitia, too. The elastic fibres in the areas with typical arterialization of the media condense intensively, form circular layers of lamellae and resemble the elastic network of the media of arteries of elastic type (Fig. 2).

Fig. 2. Transversal histological section from the wall of the cephalic vein from NAVF after 5-year functioning. 36-years old men. Abundant elastic netwoork of the wall. Orcein staining. Magn. 10 x 32.

A grossly uneven intima thickening (between 600 and 1800 μm) that to a considerable extent narrows the venous lumen is partially observed in the circumference of the vein in three cases with a period of fistula functioning of 2, 5 and 6 years, respectively. The thickening in two 20- and 36-years old subjects with periods of fistula functioning of 2 and 5 years appears as a gross expansion of the circular intimal layer, in which the cells are of various density and orientation in its separate zones. Besides, groups of radially oriented cells connecting the longitudinal intimal layer with the intimal thickening are seen in some areas with a 36-years old patient. Those areas lack elastic membrane. The layers of the membrane are not differentiated in some, or in all parts of these thickenings with the 52-years old individual with 6-years period of fistula functioning. The intimal thickenings show a layered structure probably resulting from stage expansions. It depends on the individual age and the period of fistula functioning. In the younger individuals (20- and 36-years old), they are rich in cells and fine collagen fibres, while elastic fibres lack almost completely and fine filamentous substance in some sections is detected only.

Well-developed elastic network, one or two elastic membranes, as well as formation of subendothelial membrane in

areas without subintimal membrane are observed in differentiated layers of the intima beneath them. The 6-year long functioning fistula, in contrast to them, reveals a media that becomes thin underneath as well as an intensive diluting of the smooth muscles in it and reduced elastic network - disappearing of the elastic membrane, high dilution and getting thin of the fibres, reduction of the cellular elements and increase of the collagen fibres in the intimal thickening.
The nutritive blood vessels in the venous wall are of interest. They are strongly dilated and penetrate profoundly into the wall, not only into the entire media, but also into the thickened intima and the intimal expansions as well. With a 60-years old male (8-years old fistula) they are situated in the larger part of the intima, the most far going in depth being at a distance of 70-100 μm from the lumen.

TEM
Electron-microscopically, the endothelial cells in the cephalic vein wall of functioning fistula present with many Weibel-Palade bodies and well-developed intermediate filaments. There are three SMC populations (dark, light and intermediate, with medium cytoplasmic density). Ten days after fistula functioning along with the typical SMC of contractile type without any changes there is a considerable amount of SMC of intermediate-productive-contractile type prevailing in the intima (Fig. 3).

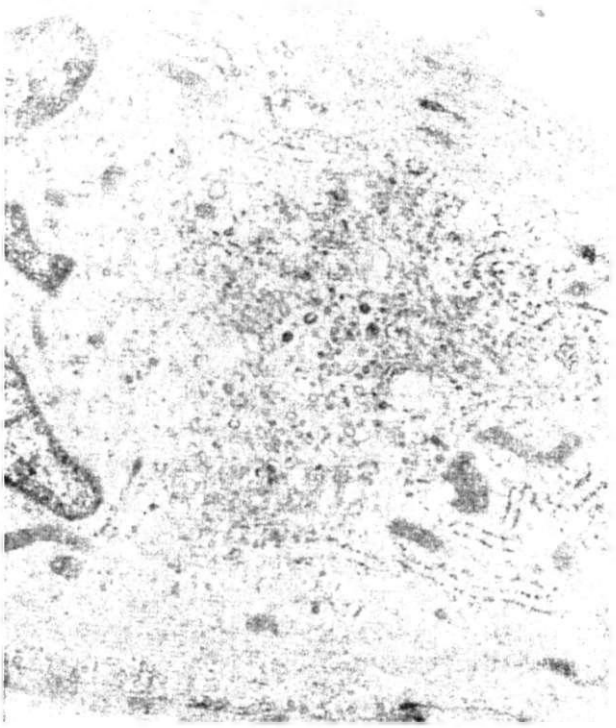

Fig. 3. SMC of secretory-contracile type. Cephalic vein from NAVF after 10 days functioning. 39-year old weman. TEM: x 12000.

These cells are rather large in size and have a well-developed organelle apparatus of secretory type: well-developed reticulum, Golgi apparatus, free ribosomes, and many coated vesicles, mitochondria and microtubules (Fig. 4). The cell organelles are situated around the nucleus as well as along the whole cell in its central part, while the actin filaments occupy the periphery. The SMC seem vacuolized after 1 year of NAVF functioning.

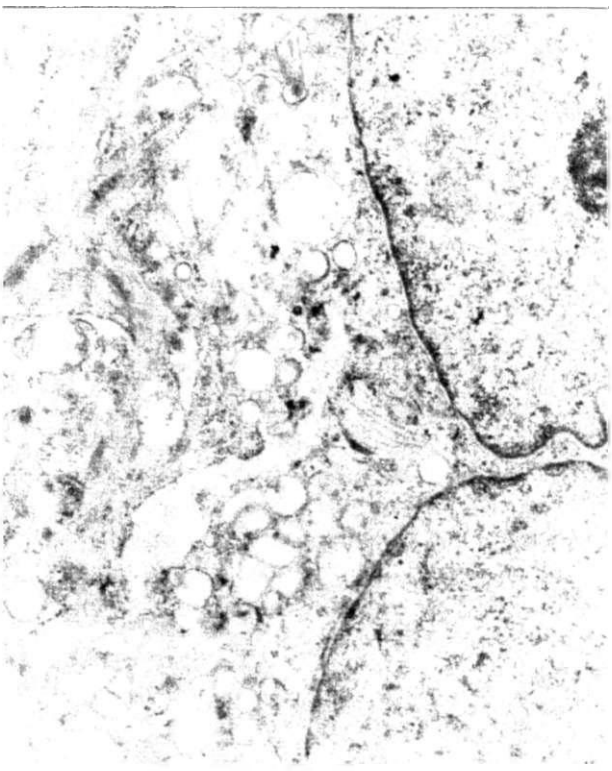

Fig. 4. Vacuolized SMC. Cephalic vein from NAVF after 1-year functioning. 53-year old man. TEM: x 12000.

Mainly around the nucleus, but at different places, spread around the sarcoplasm, optically empty spaces of various size and surrounded by largest membranes around the nucleus are observed. Single ribosomes are seen on the surface of some of them. Others possess a double membrane cover and are, probably, vacuolized mitochondria. The presence of such structures close to the sarcolema and their contact with the intracellular caveoles gives the reason for considering them as a dilated smooth reticulum.
The repeated approach to the vascular system is a key factor leading to the development of chronic healing to the renal disease. "Vascular access" for chronic hemodialysis is, respectively, one of the operations carried out most frequently in peripheral vascular surgery (9). As the number of patients treated with hemodialysis increases, the complications developing from "hemodyalisis access" failure will also increase (9). It is of significant importance to know in details the changes developing in the cephalic vein at it inclusion and functioning in NAVF in order to overcome them.
In our material the wall of *v. cephalica* included in NAVF and functioning for different periods of time is altered significantly and it differs both from the venous segments taken at the moment of creation of new fistula in uremic patients and from the wall of this vein taken from the control

material of nonuremic patients. These differences are expressed in the venous wall remodelling, in significant increase in its thickness due to thickening of both its layers - intima and media and discrete alterations in the cellular elements and extracellular matrix that build it up. These differences are most considerable in the intima and media of the wall and show certain dependence on the duration of NAVF functioning.

The intima thickness depends on the length of NAVF functioning. It increases by 3,5-4 times in fistulae that have functioned for 1 to 4 years and by more than 7 times in those ones functioning for 5 and more years. The media thickness shows the same dependence but it increases more slowly – by about two times in fistulae that have functioned for more than one year and it does not increase more significantly in those ones functioning longer. These thickenings do not relate to the advance of chronic renal failure as they are not expressed at such a degree in uremic patients with new or shortly functioning fistulae (from 1 to 10 days).

Increasing the thickness of the venous wall results from SMC hypertrophy and hyperplasia, increased quantity and thickness of collagen and elastic fibres and formation of thicker and accessory elastic membranes. During the first year of fistula inclusion these processes are more strongly pronounced in the media that is arterialized in certain areas. However, in the longer terms, changes are more outlined in the intima. In the last case not only constant thickening but also new layers which do not exist normally in this vein are seen in the intima. SMC and elastic fibres are significantly "condensed" in it. Strongly pronounced intima thickenings of different structure in certain part of the venous circumference are seen in some individuals only. They narrow the venous lumen significantly and after longer functioning they show signs of sclerosis. Initialy, SMC show increased secretory activity, while significant broadening of the smooth reticulum and almost complete degranulation of the granulated one probably due to the increased contractility are detected later on.

According to Feldman et al. (5) and Woods et al. (16), age, sex and diabetes mellitus are the major factors shortening the period of functioning of "vascular access" for hemodialysis. Peripheral vasculopathy and not diabetes, however, concern the survival of the "vascular access" (2). Venous calcification is rarely described in uremic patients. Fabbian et al. (4) report a rare case of calcification in the venous part of an arteriovenous fistula for chronic hemodialysis in a 40-years old man that has functioned successfully for 11 years, combined with calcification of the artery and an aneurysm. These authors assume that this is due to the higher blood flow determined by the higher blood pressure. There is a calcificate in the cephalic vein that has functioned for 17 years in our 47-years old man. Many calcificates and necrotic degradation in the venous neointima almost entirely obliterating the lumen are observed.

The endothelial cells of the venous wall are relatively rich in Weibel-Palade bodies. Their increase is probably due to higher pressure in the vein as such an increase is detected in endothelial cells in varicose veins (10). This augmentation of the intermediate filaments in the endothelial cells of the vein after its incorporation in the NAVF is of interest. It is possible that this leads to higher thrombogeneity in the NAVF (10). Generally, endothelial cells from out-valve regions of the venous walls are few, while the endothelium of venous valves is rich in such filaments (11). Probably, the higher pressure in the vein stimulates the development of intermediate filaments in the endothelium of the venous segment of NAVF.

Our results demonstrate a remodeling of the cephalic vein wall integrated in the NAVF. Thus it can be classified to the so-called arterialized venous wall. Anyway, in different cases, this remodeling being adaptation to the higher arterial pressure overstitches the boundaries of adaptive mechanisms and induces massive intimal thickenings accompanied by fibrosis leading to calcificates in long-functioning fistula. These alterations should be looked for using angioscopy (8), Doppler sonography (4), roentgenography (4) and other methods of investigation before reconstructive operations on the occasion of failed NAVF are carried out.

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