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PATHOHISTOMORPHOMETRIC AND IMMUNO-HISTOLOGIC CHANGES IN EARLY ARTERIOVENOUS FISTULA FAILURE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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ABSTRACT

Background: Hemodialysis is a prevalent treatment for the end-stage chronic kidney disease (CKD) worldwide. The primary arteriovenous fistula (AVF), widely considered the optimal hemodialysis access method, fails to mature in up to two-thirds of the cases. The etiology of the early AVF failure, defined as thrombosis or inability to use within three months post-creation remains less understood, and is influenced by various factors including patient demographics, surgical techniques, and genetic predispositions. Neointimal hyperplasia is a primary histological finding in stenotic lesions leading to the AVF failure. However, there are insufficient data on the cellular phenotypes and the impact of the preexisting CKD-related factors. This study aims to investigate the histological, morphometric, and immunohistochemical alterations in the fistula vein, pre-, peri-, and post-early failure.

Materials and Methods: Eighty-nine stage 4-5 CKD patients underwent standard preoperative assessment, including the Doppler ultrasound, before a typical radio-cephalic AVF creation. Post-failure, a new AVF was created proximally. The vein specimens were collected during the surgery, processed, and analyzed for morphometric analyses and various cellular markers, including Vimentin, TGF, and Ki 67.

Results: The study enrolled 89 CKD patients, analyzing various aspects of their condition and AVF failures. The histomorphometric analysis revealed substantial venous luminal stenosis and varied endothelial changes. The immunohistologic analysis showed differential marker expressions pre- and post-AVF creation.

Conclusion: This study highlights the complexity of the early AVF failures in CKD patients. The medial hypertrophy emerged as a significant preexisting lesion, while the postoperative analyses indicated a shift towards neointimal hyperplasia. The research underscores the nuanced interplay of vascular remodeling, endothelial damage, and cellular proliferation in the AVF outcomes.

Keywords: fistula failure, CKD, Vimentin, TGF, intimal hyperplasia

INTRODUCTION

Hemodialysis stands as a predominant treatment for the end-stage chronic kidney disease (CKD) globally. Despite its efficacy, vascular access dysfunction emerges as a primary contributor to the increased morbidity, mortality, and extended hospitalizations. The arteriovenous fistula (AVF),

universally acknowledged as the superior vascular access option, faces a significant challenge: approximately two-thirds of all fistulas fail to mature [1-4]. Early fistula failure is regarded AVF that is thrombosed or cannot be used for HD in the first three months after its creation. Anything later than that is regarded as late fistula failure [5]. While the pathogenesis of the late failures is relatively understood, the early failure mechanisms remain elusive, influenced by both systemic (e.g., patient's age, sex, primary renal disease, surgical proficiency, genetics) [6-9] and local factors (e.g., arterial inflow issues like atherosclerosis, stenosis, and venous outflow problems like small veins, collateral vein presence, and previous venipuncture).

Neointimal hyperplasia is the dominant histological finding that is found in significant stenotic lesions and it ultimately leads to the AVF failure, though comprehensive data on the cellular phenotype and the influence of pre-existing CKD-related factors remain sparse. Notably, oxidative stress, inflammation, and low shear stress are recognized contributors to this process. However, intimal hyperplasia is not solely responsible for the AVF failure. Emerging research highlights the inadequate venous dilatation as a crucial factor. Typically, post-fistula creation, the vein undergoes dilation and wall thickening due to the increased flow and pressure. Anomalies in this process, such as nonlinear, eccentric wall hypertrophy, result in insufficient dilation and subsequent stenosis. Biopsies from the thrombosed AVFs reveal diverse cellular components, including smooth muscle cells, fibroblasts, and myofibroblasts.

Given the complexity and the multifactorial nature of the early AVF failure, this study aims to elucidate the histological, morphometric, and immunohistochemical alterations in the fistula vein prior to, during, and following the early failure. Such insights could pave the way for improved understanding and management of the AVF in hemodialysis patients.

MATERIALS AND METHODS

A total of 89 patients with stadium 4–5 of CKD were involved in the study. A typical radio-cephalic AVF was created in all patients. The standard preoperative assessment of both extremities was performed, including physical examinations and ultrasound Doppler mapping for evaluation of vein and artery diameters. Demographic data were obtained in the time of patients referral. After AVF failed (during 90 days from its creation) a new fistula was created proximal to the one that failed.

Specimen collection and processing

During the operative procedure 8–10 mm of circumferential segment of the vein was removed near the anastomosis site. The specimen was fixed immediately in the buffered Formalin and sent to pathologist. Each tissue sample was embedded and cut into three or four blocks of 2–3 mm using the previously described technique. A semi-quantitative scoring from 0–4+ was used for the assessment of the intensity of the positive cells for the bellow mentioned markers in different parts of the venous wall (0 represents 0–10% positive cells, 1+ = 11–25%, 2+ = 26–50%, 3+ = 51–75% and 4 + = 76 = 100%). The following markers were tested: Vimentin, TGB β and Ki 67. Vimentin is a marker for the increased myofibroblasts activity and inflammation, TGB β is a marker for the oxidative stress and KI 67 is a marker for the cellular proliferation. The morphometric analysis Image J software (National Institutes of Health) was used for the morphometric analyses. The values for the luminal area (L,), intimal area (I,), medial area (M,), percentage of luminal stenosis, Intimal-medial area ratio, average and maximal intimal-medial thickness were obtained from the vein samples from each individual patient. The luminal area (L) was measured by tracing around the edge of the lumen. The intimal area (I) was measured by tracing around the internal elastic lamina, then subtracting the luminal area. The medial area (M) was measured by tracing around the external elastic lamina, then subtracting the intimal area. The intimal-medial area ratio was calculated with the formula (I-M)/(L/I). The percentage of the luminal stenosis was calculated using the formula [1-L/I] × 100. The average intimal-medial thickness (It-Mt) was obtained by averaging the measurements between the tracings used for separating both layers. The ratio of the average intimal to medial thickness was calculated with the formula It/Mt. Finally, one measurement was done from the point of the maximal intimal thickness, and then the maximal intimal-medial thickness was measured in the same way (MaxIt/Mt). By comparing the average and maximal intimal-medial thickness we can assess the eccentricity of the neointimal distribution.

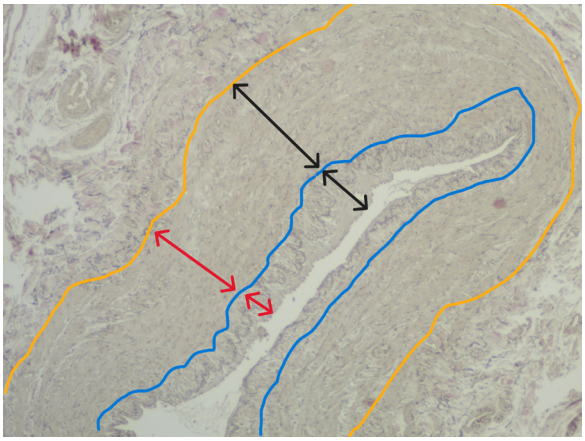


Figure 1. *Morphometric analysis. The intima is circled with blue and the media with a yellow line. The red double arrows show intimal thickness (It) and medial thickness (Mt). The black double arrows show the maximal Intimal (MaxIt) and the maximal medial thickness (MaxMt)*

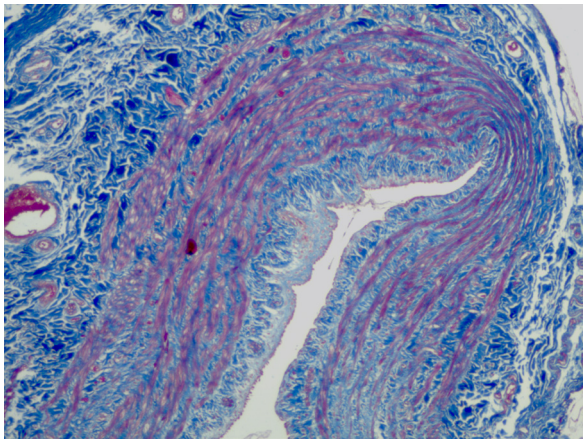


Figure 2. *Predominant medial hypertrophy. Intima is colored with blue, around it is the media with blue and pink.*

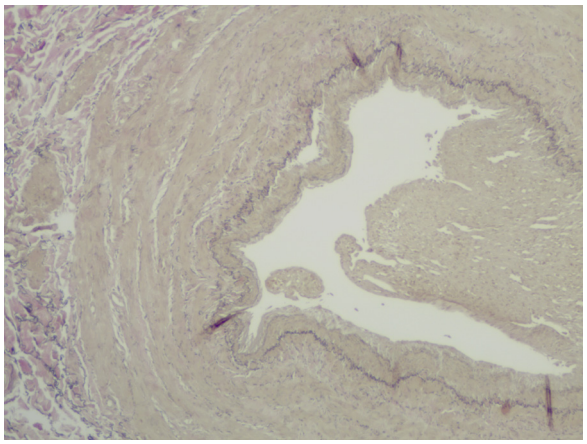


Figure 3. *Neointimal hyperplasia. Eccentric intimal hyperplasia is marked with a purple line*

Statistics

The distribution of the variables was evaluated according to the means+ SEs, medians and proportions. All statistical analysis was performed using the SPSS software package.

RESULTS

Demographics

Eighty-nine CKD patients (50 men and 39 women) were enrolled in the prospective, open, observational study. The median age was 62 ± 13 years (range 16–86). The underlying diseases were: Diabetes 20, Hypertensive Nephropathy 30, End Stage Renal Disease with unknown cause 18, Nephrolithiasis 7, Glomerulopathies 6, Adult Polycystic Kidney Disease 3, Pyelonephritis 2, Vesicoureteral Reflux 2, Vasculitis 1. All AVFs were preemptive and first vascular access.

Histomorphometric analysis

All 89 patients had adequate tissue samples for histological analysis. A substantial venous luminal stenosis (71.45 ± 23.7%) was found in most of the patients. Up to 80% of the patients showed some degree of endothelial changes at the time of the creation of the AVF, among them 19 pts with substantial intimal hyperplasia, 51 with medial hypertrophy, only 19 pts were with normal histology. The histomorphometric analyses are presented in Table 1. The values for intimal-medial area ratio and average intimial-medial thickness confirm the significant role of the medial hypertrophy in the stenotic lesions as oppose to the intimal hyperplasia. The significant difference between the maximal versus the average intimal-medial thickness suggests that the intimal lesions are eccentric.

Early fistula failure was observed in 31 patients. When we compared their results with the ones from the patients with functioning AVF, we didn't find statistical difference in the histomorphometric analysis.

The majority of the patients with failed AVF had marked medial hypertrophy as opposed to the intimal hyperplasia (21:10) before the fistula was created.

After the creation of the new AVF, the dominant finding was increased in the intimal hyperplasia in both subgroups, which was statistically significant in the subgroup with previous marked medial hypertrophy, and without a statistical significance in the group with intimal hyperplasia (Table 1).

Immunohistologic analysis

There is a various extent of expression for the markers. Even before the creation of the AVF, there was an increased expression in Vimentin (Table 2), but insignificant of TGB β, in both groups (with functioning and with failed AVF) (Table 3). In the second group, after the failure, the expression of both markers has risen in both subgroups, but not statistically. We found insignificant expression of KI 67 both, before and after the fistula failure.

Table 1. *Intima-media thickness ratio*

	Intimal hyperplasa- (intima/media thickness ratio)			
	N	mean ± SD	median	min - max
observation point 1	10	0,33±0,2	0,31	0,1 – 0,55
observation point 2	10	0,35±0,2	0,305	0,1 – 0,63
	Wilcoxon Matched Pairs Test Z = 1,58 p = 0,11 ns			

	Medial hypertrophy- (intima/media thickness ratio)			
	N	mean ± SD	median	min - max
observation point 1	21	0,17±0,1	0,15	0,05 – 0,39
observation point 2	21	0,21±0,1	0,20	0,06 – 0,54
	Wilcoxon Matched Pairs Test Z = 3,28 p = 0,001**			

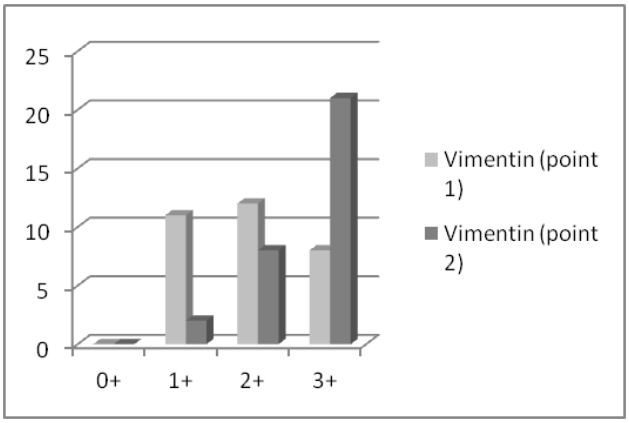


Figure 4. *Vimentn expression (prior and after the AVF failure)*

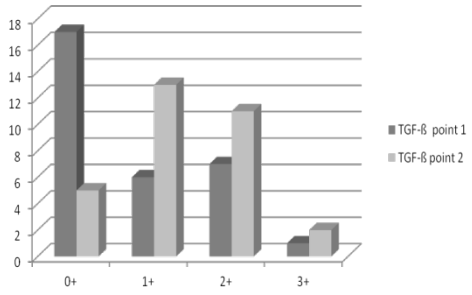


Figure 5. *TGF β expression (prior and after the AVF failure)*

DISCUSSION

In our study we present 89 patient with CKD stage 4/5 in whom we have created preemptive AVF. Our analysis encompasses histomorphometric and immunohistochemical evaluation of the native vein segments used for the AVF construction. The early AVF failure, observed in 31 patients within 90 days post-creation, led to the construction of a secondary fistula proximal to the same vessels.

The underlying conditions such as hypertension, advanced ESRD, diabetes, age, and gender might predispose patients to the preexisting endothelial changes, potentially influencing the vascular response.

Our immunohistochemical analysis targeted three biomarkers: Vimentin (indicative of myofibroblast activity and inflammation), TGF-β (marker for oxidative stress), and KI-67 (cellular proliferation marker). At the initial AVF creation, the Vimentin expression was absent in only 5% of patients with functioning AVFs. The majority exhibited significant expression (50-75%), with no statistical differences between patient groups. TGF-β predominantly scored 0 (0-1% expression) across most patients, with a slight, statistically insignificant dominance in the test group. KI-67 expression was negligible or absent. Post-early failure analysis revealed increased, but not statistically significant, expressions of Vimentin and TGF-β.

The histomorphometric analysis revealed a spectrum of preexisting venous endothelial changes. The intimal-medial area ratio and the average intimal-medial thickness (Table 1) indicate a predominance of medial hypertrophy in the stenotic lesions. This was corroborated by the vein sample histology, with 51 patients showing predominant medial hypertrophy and 19 exhibiting intimal hy-

perplasia. Early fistula failure was nearly equally prevalent in both subgroups. Notably, varying degrees of intimal stenosis (5-95%) were observed at fistula creation, despite the absence of significant stenosis in the preoperative Doppler ultrasound mapping. The post-failure analysis showed a marked increase in the venous stenosis and the neointimal hyperplasia, even in veins initially exhibiting significant medial hypertrophy. The maximal to average intimal-medial thickness ratio indicated eccentric neointimal stenotic lesions.

This data suggests that neither medial hypertrophy nor intimal hyperplasia alone can predict early fistula failure, although neointimal hyperplasia may contribute to it. The vascular remodeling (positive vs. negative) also appears to play a role. The AVF creation, while inducing increased flow, has been linked to low and intermittent flow regions, particularly juxta anastomotic, where most AVF stenoses occur [10]. This leads to heightened protease activity [11]. Fibroblast migration from the adventitia through the media, acquiring SMA expression and transforming into myofibroblasts, as well as the transformation of the contractile muscle cells from the media into the intima, are hypothesized mechanisms [12-14]. The presence of these cells suggests active processes influencing neointimal hyperplasia and subsequent AVF stenosis.

TGF-β1 expression correlates with the neointimal hyperplasia and the local inflammatory processes, playing a key role in cell proliferation and differentiation [15-19]. Its local synthesis by smooth muscle cells in the media and intima [18] and its effect on the intimal thickening, as demonstrated in animal models [20], underline its significance. The lack of TGF-β expression pre-AVF creation and its subsequent increase suggest that inflammation and oxidative stress are consequences of the vascular access surgery.

In conclusion, endothelial damage in advanced ESRD may contribute to the future fistula failure. Our findings indicate that medial hypertrophy is a predominant preexisting pathohistological lesion, alongside neointimal hyperplasia. The absence of TGFβ expression prior to the AVF creation and its later increase imply post-surgical development of inflammation and oxidative stress. The increased presence of myofibroblasts and fibroblasts (Vimentin positive cells) in the media and the intima suggests a predisposition in some patients to venous endothelial changes, possibly as a response to uremic, hypertensive, and other insults.

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Резиме

ПАТОХИСТОМОРФОМЕТРИСКИ И ИМУНОХИСТОЛОШКИ ПРОМЕНИ ПРИ РАНО ОТКАЖУВАЊЕ НА АРТЕРИОВЕНСКИ ФИСТУЛИ КАЈ ПАЦИЕНТИ СО ХРОНИЧНА БУБРЕЖНА БОЛЕСТ

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Вовед: Хемодијализата претставува најчесто користен метод на третман кај пациентите со хронична бубрежна болест (ХББ) во терминален стадиум. Примарната артериовенска фистула (АВФ), иако се смета како оптимален пристап за хемодијализа, нематурира во две третини од случаите. Етиологијата на рано откажување на АВФ е недоволно разјаснета и се смета дека различни фактори, вклучително генетските и демографските фактори, хируршката техника и сл., имаат свое влијание. Неоинтимална хиперплазија е најчест хистолошки наод при стенозирани лезии, кои водат до откажување на АВФ. Сепак, сè уште нема доволно податоци за клеточниот фенотип и влијанието на преегзистирачките фактори поврзани со самата болест. Целта на оваа студија е да ги испита хистолошките, морфометриските и имунохистохемиските промени, кои се јавуваат во фистулните вени пред откажувањето на АВФ, како и за време и по откажувањето од него.

Материјали и методи: Кај 89 пациенти со ХББ ст. 4/5, по направената стандардна преоперативна процедура, вклучително и преглед со УЗ-доплер, пред да биде направена типична радиоцефалична АВФ. При нејзиното откажување, проксимално беше креирана нова фистула. Во текот на интервенцијата дел од вената беше земен, обработен и испратен за морфометриска анализа, како и испитување на клеточната експресија на неколку маркери: Виментин, ТГФ, КИ67.

Резултати: Во оваа студија беа вклучении 89 пациенти со ХББ, кај кои беше направена анализа на факторите што би довеле до рано откажување на АВФ. Хистоморфометриската анализа покажа значајна венска луминална стеноза и промени во ендотелното ремоделирање од доминатна медијална хипертрофија пред креирањето на фистулата, кон доминација на ендителната хиперплазија по нејзиното откажување. Имунохистолошката анализа покажа значајна експресија на Виментин, како пред креирањето така и по раното откажување на фистулата, како и зголемена експресија на ТГФ бета по нејзиното откажување.

Заклучок: Оваа студија ја потврди комплесноста на раното откажување на АВФ кај пациентите со ХББ. Предоперативно се детектира медијална хипертрофија, но по откажување на фистулите се забележува зголемена интимална хиперплазија. Исходот на АВФ зависи од интеракцијата меѓу васкуларното ремоделирање, ендотелното оштетување и клеточната пролиферација.

Клучни зборови: откажување на фистули, ХББ, Виментин, ТГФ бета, интимална хиперплазија