Short Thesis for the Degree of Doctor of Philosophy (PhD)

Hemodynamic and Microcirculatory Effects of a Microsurgical Experimental Carotid-Jugular Fistula

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University of Debrecen
Doctoral School of Clinical Medicine
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The PhD defense takes place at the Lecture Hall of the Building 'A' of the Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 13:00, 8th of September, 2021.

1. INTRODUCTION

Arteriovenous fistula (AVF) is a significance modification of the cardiovascular system since it is an early connection between the arterial and venous circulations, thus the blood flows directly from the artery to the vein bypassing the distal circulation. The AVF is very common in the clinical practice, since it could be congenital as arteriovenous malformation (AVM) or acquired as hemodialysis fistula, iatrogenic fistula and traumatic fistula.

Congenital AVFs are rare and exist usually due to the failure of the differentiation of embryonic vessels into arteries and veins. Hemodialysis fistulas provide long-term, high-flow, and easy access for hemodialysis patients without any real alternatives. Therefore, these fistulas are the most common form of AVFs. The incidence of iatrogenic AVFs is increasing exponentially due to increased percutaneous interventions and the femoral AVFs are the most frequent form of these fistulas. Traumatic AVFs occur in the civilian and military vascular trauma, and can be found in all vessels, especially in the neck and abdomen. Spontaneous AVFs should also be also mentioned, they occurred after the rupture of diseased artery into a contiguous vein.

Arteriovenous fistula has been and is still the focus of many studies due to the wide spectrum of systemic effects. The systemic effects are not limited to the cardiovascular system, as they involve other neurohormonal and pulmonary effects such as pulmonary hypertension and the changes in natriuretic peptides. Patients with arteriovenous fistula face in addition to the systemic complications many other local ones as local venous hypertension and mass effects of the fistula.

On the other side, many challenges exist to maintain the patency of the arteriovenous fistula as the only reasonable vascular access in hemodialysis patients. These challenges include recurrent stenosis, thrombosis of the arteriovenous fistula, impaired maturation, access-related complications and intimal hyperplasia.

The fistula-related hypoperfusion or ischemia is one of the important complications. The arteriovenous fistula impairs the distal circulation, and this impairment could lead in 1-9% of cases to critical access-related ischemia. It is challenging to manage this kind of ischemia. Although it could actually be managed by the surgical closure or banding of the fistula, but in the case of hemodialysis fistulas, the main challenge is to manage these complications without sacrificing this important hemodialysis access.

Numerous medical studies have been conducted to figure out new aspects that can be used for the management of this hypoperfusion, we intended by this study to approach the fistula-related hypoperfusion from a microcirculatory point of view and to identify the factors affecting the microcirculation. Many animal models were used to study the pathophysiological effects of the AVFs. There is no complete model simulating AVFs in humans, and each model has many disadvantages and advantages. According to our Knowledge, a model to study the microcirculation and rheology has not yet been evaluated. A carotid-jugular fistula (CJF) model will be tested in this study as a feasible model for such studies.

2. Objectives

1. The main aim of this study was to evaluate another mechanism of fistula-related ischemia at the microcirculatory level. Our hypothesis was that the high turbulent flow through the arteriovenous fistula worsens the micro-rheological properties of RBCs as a suggested mechanism of impaired tissue perfusion.
2. This study was also conducted to check the feasibility of carotid-jugular fistula (CJF) as a model to study fistula-related microcirculatory and systemic changes, thus a feasible model to test our hypothesis.

3. Another purpose was to show the effect of arteriovenous fistula on the hemodynamic and morphology of remote organ as well as to compare our results with other fistula models in rats.

3. Materials and methods

3.1. Experimental animals

The experiments were done at the Department of Operative Techniques and Surgical Research and registered by Committee of Animal Welfare at the University of Debrecen, (Registration Nr.:25/2016/DEMÀB) according to the Hungarian Animal Protection Act Law XXVIII/1998, EU Directive 2010/63/EU and the Ordinance 40/2013.

Wistar (Crl:WI) rats were used in this study, the rats were healthy female at 10-12 weeks of age. The total number of experimental animals was sixteen, ten rats in the fistula group with an average weight of 272±22 g (FG, n=10) and six rats in the sham-operated group with an average weight of 267±11 g (SG, n=6).

The anesthesia protocol included the administration of sodium thiopental (60 mg/kg, intraperitoneal, Thiobarbital 0.5 g, B. Braun Medical S.A., Spain) and Atropine (0.05 mg/kg subcutaneous). All stages of follow-up were done by the same anesthesia protocol.

3.2. Operative techniques

The surgery was done in sterile conditions. An oblique surgical incision, about 1.5 cm in length, was made at the lower part of the neck just above the right clavicula and the sternal notch. The right external jugular vein (EJV) was isolated carefully to its large tributaries i.e. for about 7 mm above the clavicle. After enough preparation of the vein, the right common carotid artery (CCA) was isolated medial to the sternocleidomastoid muscle (SCM) for about

10-12 mm above the clavicle. The distal ends of both artery and vein should be ligated. The proximal parts of both the artery and vein were controlled using fine clamps. The artery was cut a 45-degree angle medially and obliquely so that the artery diameter matched to the diameter of the vein. The EJV was cut perpendicular under the ligation. An end-to-end anastomosis was constructed in front of SCM between the proximal ends of artery and vein with continuous suture using 10-0 polyamide suture material (Silon, Chirmax, Prague, Czech Republic). The clips were released after the construction of the anastomosis to check any bleeding from the anastomosis. The skin was closed with continuous suture using a 4-0 Glycolide-ε-Caprolactone (Monolac, Chirmax, Prague, Czech Republic).

The blood loss was replenished using normal saline solution subcutaneously to, and Flunixin (2.5 mg/kg, s.c.) was administered after the surgery for pain management. The rats were returned after recovery to individual cages. They were then subjected to regular monitoring as well as wound care.

The rats in the SG were subjected to the same surgical protocol in terms of preparation, surgical incision and vessels isolation, but without any further other vascular interventions such as ligation, clamping, or anastomosis.

This experiment was done under an operating microscope (Leica Wild M650, LEICA Ltd., Germany), with documentation be photos and video. The diameter of CCA and EJV was measured by a scaled clip, the diameters were in average 1 and 1.5 mm respectively.

3.3. Follow-up protocol

The follow-up period was 6 weeks and was determined after reviewing the literature to achieve the goal of the study by evaluating the isolated effects of arteriovenous fistula, in other words before the developing of heart failure, approximately 8 weeks. The measurements were carried out before the construction of the fistula as baseline measurement (FG-base and SG-base) and one week after the surgery (FG-1W and SG-1W) and six weeks after the surgery (FG-6W and SG-6W).

The measurements included the following: neurobehavioral assessment, evaluation of the patency of the fistula, evaluation of the microcirculation by laser Doppler, hemodynamic measurements, weight measurements, hematological and hemorheological measurements. The rats were sacrificed by exsanguination at the end of the measurements, i.e. at the 6^{th} week follow-up, then we have taken tissue samples for histologic studies.

3.4. Neurobehavioral assessment

Garcia scale was used for the neurological examination of these rats. This scale includes six components: spontaneous activity, symmetry in the movement of limbs, forepaw extension, climbing, body proprioception and response to vibrissae touch. The maximum score for each component is 3 and the minimum score is zero or one, and the minimum overall score is 3 and the maximum is 18.

3.5. Evaluation the patency of the fistula

After the operation, the fistula was checked by milking test (clamping the vein after the anastomosis with first forceps then emptying the vein of blood by squeezing the vein with second forceps for a few millimeters from the first forceps. The fistula is patent when the vein refills quickly after the releasing of the first forceps).

The patency of AVFs was confirmed at the first week follow-up by the physical examination and handheld Doppler. At the sixth week follow-up, the fistula was isolated through a small incision to check the patency by the pulsatile appearance and milking test.

3.6. Hemodynamic measurements

Hemodynamic measurements in our study included measurement of systolic and diastolic pressure and heart rate. Two systems were used in these measurements, non-invasive and invasive system. The non-invasive system was used for the baseline measurement and 1st week follow-up. The method of measurement was a non-invasive tail cuff method by using a non-invasive Blood Pressure Amplifier (Apollo, IITC Inc., CA, USA).

The invasive System was used at the 6th week follow-up by placing the catheter in the abdominal aorta. The used device was (LD-01, Experimetria Ltd., Hungary), and G20 cannula was used as catheter. The hemodynamic parameters were measured before the occlusion of the fistula or the CCA, the same measurements were repeated once again after the acute occlusion of the arterial limb of the fistula in FG or the CCA in the SG.

3.7. Laser Doppler measurements

This microcirculation was evaluated by the laser Doppler, and this evaluation was carried out by the recording of laser Doppler waves from the organs distal to the fistula, in other words the microcirculation of the liver and kidney. We have chosen these organs since they are the first suitable and measurable organs distal to the origin of the fistula. In addition to the availability of the required expertise and tools for these measurements at our department.

The microcirculation was evaluated only at the end of the experiment in both groups, since it needs an invasive procedure, a laparotomy. These measurements were done twice, once before the occlusion of the fistula and once after the occlusion of the arterial limb of the fistula in FG or the CCA in SG. The laser Doppler (LD) tissue flowmetry (LD-01, Experimetria Ltd., Hungary) was used with a standard pencil probe (Oxford Optronix Ltd., UK). S.P.E.L. Advanced Kymograph software (Experimetria Ltd., Hungary) was used to record the measurements and analyze the values of the waves. The values of the waves were expressed in Blood Flux Units (BFU [au]).

3.8. Blood sampling

The blood samples were venous at all stages and were taken without using of tourniquets. The used veins were the lateral tail veins, right external jugular vein, and caudal caval vein. BD Vacutainer® tubes were used (K3-EDTA 5.4 mg / 3 ml). The laboratory measurements were begun within 30 minutes after blood sampling.

3.9. Hematological measurements

Hematological parameters were tested using (Sysmex F-800 automate, Sysmex Co., Ltd. Japan). The measurement requires about 70 μl of blood. The studied parameters were: white blood cell count (WBC [10^3 / μl]), red blood cell count (RBC [10^6 / μl]), hemoglobin (Hgb [g / dl]), hematocrit (Hct [%]), mean corpuscular hemoglobin (MCH [pg]), mean corpuscular volume (MCV [fl, Femtoliter]), mean corpuscular hemoglobin concentration (MCHC [g / dl]), red blood cell size distribution (RDW-CV%), platelet count (Plt [10^3 / μl]) and mean platelet volume (MPV [fl]).

3.10. Hemorheological measurements

The RBC deformability was evaluated using laser diffraction ektacytometry technique. This technique allows us to measure the elongation of the RBCs shape at a specific shear stress using a laser diffraction pattern. LoRRca MaxSis Osmoscan (Lorrca®) is used in our department, manufactured by Mechatronics BV in the Netherlands (Lorrca is the acronym for Laser Optical Rotational Red Cell Analyzer). The RBCs are exposed to increased shear stress

(SS), the elongation of the RBCs is measured at different shear stresses in the range from 0.3 to 30 Pa. The data are presented as EI-SS curves.

Each case is represented by a curve. We compare these curves by comparing some parameters calculated from the curve, the maximum elongation index (EI_{max}), the shear stress corresponding the half of the maximum elongation index ($SS_{1/2}$ [Pa]) and the ratio between EImax and SS1/2. These parameters are calculated using Lineweaver-Burke analysis ($1/EI = (SS_{1/2}/ EI_{max}) \times (1/SS) + (1/EI_{max})$).

The mechanical stability of the membrane was evaluated by subjecting the RBCs to a high mechanical pressure (100 Pa, 300 s) and comparing the deformation of the RBCs before and after. The above-mentioned parameters (EI values, EI_{max} and $SS_{1/2}$) were compared and the ratio between before and after was calculated.

3.11. Density separation of RBC subpopulations

We assumed that more information will be available from studying the hemorheological differences between RBCs subpopulations, since the AVF could influence on the old RBCs populations through some mechanisms such as intravascular hemolysis.

The measurements of density separated RBCs require 2-3 ml of blood. This amount of blood is quite large relatively to rats, so these measurements were performed on other six healthy female rats as a negative control group (CG, n=6, bodyweight: 271.33±11 g) and on 5 rats of FG-6W. About 2-3 ml of blood was drawn from the caudal caval vein, and hematological and hemorheological measurements were done. The rest of the sample was centrifuged at 4000 rpm (1800 g) for 60 min using centrifuge (Hettich Universal 32R, Germany), the layer of clear fluid (plasma) was aspirated and kept aside, and the 'buffy-coat' was removed. Since rat blood cells are known to be sensitive for physical effects, we did not wish to use centrifugation at higher force.

Ten percent of RBCs was calculated depending on the base values of Hct and sample size. This percent was aspirated from about the top 10% and bottom 10% of the RBC column. Top RBCs were considered as dominantly ‘young’ RBCs and bottom RBCs were considered as dominantly ‘old’ RBCs. Top and bottom samples were diluted in autologous plasma adjusting the Hct to 40%.

3.12. Hematocrit standardization

The effect of hematocrit on rheological parameters has been the focus of several studies. Here we intended to conduct a more in-depth study of the results of the previous step to find out the effect of hematocrit on the RBCs deformability.

The samples of the young and old RBCs were considered a representative of standardized Hct in both groups, where the hematocrit value is 40%, and the RBCs deformability was measured in these samples. The RBCs deformability was measured in the native samples before hematocrit standardization as well. The RBCs deformability was then compared between before and after Hematocrit standardization.

3.13. Weight measurements

Body weight was measured at each stage of the study (Base line, 1st and 6th week postoperative). The weights of the organs (heart, lungs and liver) were measured only at the 6th week follow-up at the end of the experiments. The relative weight of the organs was calculated by dividing the absolute weight of organs by the body weight and used to compare the groups. Organs weight was measured by using Analytic scale (LMIM, LB-1050, Hungary).

3.14. Histological examinations

Tissue samples were taken at the end of the experiment and fixed in 4% buffered formaldehyde solution. These samples included the excision of the hearts and the vessels mentioned later. Histology slides were scanned using the following device (Pannoramic MIDI II, 3DHISTECH Ltd, Hungary) and this software (Pannoramic Viewer, 3DHISTECH Ltd, Hungary) was used to for the measurements.

The sections of the Hearts were stained using Hematoxylin and Eosin (H&E). The thickness of the left ventricular wall was measured in 4 different points and the thickness of the right ventricular wall was measured in 3 different points.

Vessels samples were also taken to compare the intima thickness of the venous limb of the fistula with the contralateral vein (non-operated EJV in FG) and with the vein in SG (right EJV). Elastic Van Gieson (EVG) was used to stain these sections. The thickness of the intima was measured in ten points and the mean of highest three measurements for each sample was considered as intima thickness.

3.15. Documentation sheet

All the data obtained from the experiments was documented in one application, such as vital parameters, operation notes, neurological assessments and the medications. All stages of the experiment were documented in the same form.

3.16. Statistical analysis

The results were expressed as means ± standard deviation (SD). For normally distributed data, the paired samples T test was used to compare two paired samples and the one-way ANOVA test was used to compare two independent samples. For non-normally distributed data, the Wilcoxon signed-rank test was used to compare two paired samples and the Mann-Whitney U test was used to compare two independent samples. $P<0.05$ was considered statistically significant. IBM SPSS Statistics version 22 was used for the statistical analysis and Microsoft Excel 2016 was used for designing the graphs.

4. Results and discussion

4.1. Surgical Procedure

Hemodialysis arteriovenous fistulas are performed using different types of anastomosis such as side-to-end, end-to-end or end-to-side anastomosis, and using many techniques such as suturing technique, puncture technique or cuff technique. The fistula can be established in peripheral or central vessels. We chose suturing technique to perform this anastomosis between two relatively large peripheral vessels, as our goal was to follow the rats for a relatively long time so a safe anastomosis with minimal complications was needed. The two golden rules for creating our anastomosis were to perform an anastomosis without any tension on the anastomosis line and to work in a moist environment.

The superficial location of the CJF allowed us to check fistula patency without invasive interventions, in contrast to central arteriovenous fistulas. The superficial location of a jugular carotid fistula simulates the superficial location of the hemodialysis arteriovenous fistulas in humans and provides a model for studies about local treatments or local measurements such as measuring local microcirculation, unlike the aortocaval fistula, one of the most common types of fistula used in studies.

4.2. General observations

The complication in our study was few, one rat died of uncontrolled arterial hemorrhage in FG. No other surgical complications occurred in our study. Studies have reported different mortality rates ranging from 0 to 47%. The mortality rate is expected to be higher in central arteriovenous fistulas where surgical trauma is greater, and the risk of bleeding and concomitant infection is greater.

Puncture technique is a simple and fast procedure, but it is not possible to do it in the neck region since the internal carotid artery and the internal jugular vein are not adjacent vessels. We used suturing technique to perform the anastomosis to reduce the risk of hemorrhage and death, and all the anastomoses were performed in less than 45 minutes.

4.3. Neurobehavioral assessment

Based on Garcia scale, the mean base neurological score was 17.33±0.82 for sham rats and 17.66±0.5 for fistula rats. The rats in both groups were revaluated after 6 weeks of the surgery by the same scale, and the mean neurological score was 17±0.89 for sham rats and 17.44±0.88 for fistula group. Neurological evaluation using the Garcia scale showed that no significant neurological deficit occurred in both groups. This supports the medical literature that unilateral carotid artery ligation in rats does not cause significant neurological deficit, unlike the case in humans with carotid artery ligation.

The most important clinical sign in the neurological examination was the drop of the right upper eyelid in about half of the cases, 4 cases in FG and in 3 cases in SG. We interpreted the dropping of the right upper eyelid as part of Horner's syndrome especially it happened in both FG and SG rats. Horner's syndrome is a well-known syndrome after carotid artery surgery, whether in humans or in rats.

4.4. Evaluation the patency of the fistula

The patency of the fistula was checked during the experimental stages, whether by physical examination, by using the handheld Doppler, or by surgical isolation. There was no case of fistula occlusion during the study stages.

4.5. Hemodynamic measurements

In non-invasive measurements, the baseline value of SBP in FG was 107.83±33.25 mmHg and significantly decreased ($p=0.041$) to 76.83±8.64 mmHg one week after the construction of the fistula. The invasive measurements showed also significant decrease ($p<0.01$) in SBP in FG (107.02±27.52 mmHg) in comparison with SG (145.17±13.09 mm Hg); The DBP is also significantly decreased ($p<0.01$) in FG (70.39±24.06 mmHg) in comparison with SG (117.59±13.72 mmHg).

The CJF model in our study showed expected hemodynamic effects by both non-invasive measurements at the beginning of the study and invasive measurements at the end of the study.

The non-invasive measurements showed that HR in FG was 345.67±20.91 min^{-1} one week after the construction of the fistula and this value was significantly lower ($p=0.026$) in comparison with the baseline value 402.5±29.12 min^{-1}. The invasive measurement showed also marked decrease of the HR in FG in comparison with SG without reaching the significant level.

As a conclusion, we could appreciate significant decrease in HR by using both invasive and non-invasive measurements. The arteriovenous fistula leads to an additional load on the heart, so it leads to increase the cardiac output in human by various mechanisms, including an increase in the heat rate. The mechanisms of increasing cardiac output in rats may be different from those in humans and depend on the decrease in the heart rate to allow the heart to get sufficient venous return between the two heartbeats since the heart rate in rats is very fast compared to humans and reaches normally 300-400 beats per minute.

The hemodynamic status changed significantly after the acute occlusion of the AVF. The invasive measurements showed immediate and significant increase in both SBP and DNP ($p<0.01$) after the acute occlusion of the fistula. No difference was observed in the HR after the occlusion in both groups. The measured values in the SG did not differ significantly after the acute occlusion of the CCA.

The sharp increase in both SBP and DBP is due to several physiological mechanisms, including the sharp rise in peripheral vascular resistance. The heart rate did not change significantly after the acute fistula occlusion, and this may be due to the effect of atropine given preoperatively.

4.6. Laser Doppler measurements

According to our information, there is a lack of studies evaluating the microcirculation in the tissues and organs distal to the fistula. Therefore, we present our model as a AVF model with the ability to perform a microcirculation study.

The liver BFU values were significantly lower ($p=0.01$) in FG (8.78±3.16) in comparison with SG (14.44±3.76). However, these values increased significantly once again after the acute occlusion of the fistula ($P<0.01$) to reach this value (18.37±6.46). As opposed to the liver, no significant difference of the kidney BFU values was observed between FG (21.73±4.49) and SG (17.52±4.56), and the kidney BFU values increased after the acute occlusion of the fistula to this value 26.08±3.71 but without reaching the significant level. The BFU units of both kidney and liver didn´t change significantly after the occlusion of the CCA in SG.

The hepatic microcirculation showed results comparable to the hemodynamic results. The renal microcirculation did not show significant changes in the values of these parameters, neither between the two study groups nor after the acute occlusion of the fistula, despite the important hemodynamic changes. These results refer to the ability of the kidney to self-regulate the local blood flow.

4.7. Hematological measurements

RBC count and Hct were increased significantly in FG-6W in comparison with both SG-6W ($p<0.01$) and FG-base ($p<0.001$). We have interpreted the increased measured erythrocyte mass in fistula rats due to secondary polycythemia induced by excess erythropoietin in the blood. Studies have not shown consistent results about the effect of arteriovenous fistula on hematocrit values.

Studies have not shown consistent results about the effect of arteriovenous fistula on hematocrit values, as some studies showed a significant decrease in the hematocrit value in contrast to our study but by using an aortocaval model. The location of the arteriovenous fistula may play a role in interpreting these results, as the liver and the kidney are the distal organs in our model, and they are both the most important producers of erythropoietin in the body, while in the case of aortocaval model and saphenous model, the distal organs are usually the lower extremities and the pelvic organs.

RBC indices showed also hypochromic RBCs and wider distribution in RBC size in FG-6W in comparison with FG-base, as MCH decreased significantly ($p=0.002$) from 15.8 ± 0.7 in FG-base to 13 ± 2.2 in FG-6W and RDW-CV increased significantly ($p=0.007$) from 13.09 ± 0.54 in FG-base to 13.76 ± 0.7 in FG-6W. These results can be explained indirect signs of iron deficiency. We did not determine the serum iron levels in our experiment because it was not included in the study protocol at the beginning. The assumed iron deficiency could be due to depletion of iron stores to support erythropoiesis.

The platelet count increased gradually during follow-up in the fistula group, while the mean platelet volume (MPV) decreased. These changes could be interpreted as an accompanied iron deficiency, but there are other differential diagnoses for thrombocytopenia in this case such as acute phase reactions and spurious increase related to fragmented RBCs.

4.8. Red blood cell deformability

The $SS_{1/2}$ and $EI_{max}/SS_{1/2}$ were calculated from the EI-SS curves. No significant difference in baseline values of these two parameters was observed between the two study groups. The $EI_{max}/SS_{1/2}$ was significantly lower in FG at 1 week (FG-1W, p=0.025) and 6 weeks (FG-6W, p=0.039) compared to parallel values from the SG (SG-1W and SG-6W). While the $SS_{1/2}$ value was significantly higher in FG at 1 week (FG-1W, p=0.003) and 6 weeks (FG-6W, p=0.043) compared to parallel values from the SG (SG-1W and SG-6W).

The overall evaluation of the rheological parameters showed a statistically significant decrease in the RBCs deformability in FG compared to the SG in both the first- and sixth-week measurements.

The individual evaluation of the RBCs deformability parameters showed a significant difference between the FG rats, as some rats did not show a significant change in the RBCs deformability parameters, while others showed significant drop in these parameters. These large individual differences occurred even though all these rats were of the same gender, strain, and age and had the same experimental environment. These individual differences explain the high value of SD.

4.9. Red blood cells membrane stability

After applying membrane stability test (100 Pa shear stress for 300 s) on RBCs, the deformability of RBCs was decreased significantly (p<0.01) in SG-6W for shear stress range (0.95-30 Pa) and in FG-6W for shear stress range (3-30 Pa). The ratio of RBC deformability values after/before applying the stress was calculated at each shear stress and compared between FG-6W and SG-6W. This ratio was smaller in FG-6W, however, without significant differences.

4.10. Comparing young and old RBCs deformability

After the separation of the subpopulations of RBCs, no significant differences were observed in hematological parameters except significant increase ($p=0.014$) of RDW-CV in the bottom 10% of FG-6W in comparison with the top 10% of FG-6W. Top 10% RBCs showed slightly higher deformability than bottom 10% RBCs without the expected significant differences between the subpopulations. Maybe we had to centrifuge using higher forces or longer time as in other studies (10000 × g for 15 min).

4.11. The effects of hematocrit standardization

The measured parameters ($SS_{1/2}$ and $EI_{max}/SS_{1/2}$) in the CG did not show a significant statistical difference between the results before and after the hematocrit standardization, but these parameters showed a significant improvement in the RBCs deformability after the hematocrit standardization. The same significant differences were not observed in CG, since the change of Hct in CG was only 7% significantly less than the Hct change in FG (approximately 19%).

4.12. The weight of body and organs

The mean body weight of SG rats was 267.17±11.18 g and they gained weight gradually to 287.5±15.45 g six weeks after the surgery, while the body weight of FG rats decreased slightly one week after the construction of the fistula and they regained the weight to 294.57±29.2 g at the end-point.

The loss of weight at first week in FG may be due to the post-operative stress, many studies showed same temporal loss of body weight after the surgery. The rats in FG regained the weight after 6 weeks without a pathologic significant changes, since rapid body weight gain after fistula surgery is an evidence of fluid retention, and some studies have considered an increase of 50 grams or more within 7 days as evidence of severe heart failure.

The mean absolute and relative weights of the hearts were increased significantly in FG compared with SG. Heart weight gain is an evidence of cardiac hypertrophy, and many studies considered that a ratio of heart weight to body weight more than 5 mg / g is an indicator of severe congestive heart failure. The heart weight to body weight ratio in our study was 4.45±0.39 mg/g.

No significant difference was observed in lung weight to body weight ratio between FG and SG. Lung weight more than 2.5 g is also an indicator of severe congestive heart failure. mean lung weight was in our study 1.4 g.

Finally, it is important to mention that the effect of an arteriovenous fistula on the weight of the organs varies according to the size of the fistula.

4.13. Histological examinations

The measurements showed significant left ventricular hypertrophy (LVH) in FG since wall thickness values were increased significantly ($p=0.008$) in FG (3.218±0.7 µm) in comparison with SG (2.425±0.7 µm), but no significant difference was observed in right ventricular wall thickness between the groups. Intima proliferation was observed in the venous limb of the fistula and highly significant increase ($p<0.001$) in the thickness of the intima of the venous limb of the fistula (84.56±26.8 µm) was observed in comparison with non-operated EJV in FG (5.37±1.2 µm) and EJV in SG (3.8±0.69 µm).

The arteriovenous fistula in this model resulted in statistically significant left ventricular hypertrophy compared to the control group due to overloading via the arteriovenous fistula. All these data and the high survival rate refer to significant cardiac pathology induced by CJF but without reaching a severe congestive heart failure at the end-point measurement.

We would also recommend this model for studies on intimal hyperplasia in the venous section and its management.

5. Main findings

- The Carotid-jugular fistula resulted in a significant increment of RBC mass and a significant impairment of RBC deformability. These changes could be one of the pathways through which the fistula influences the microcirculation, which opens the door for further studies on a novel proposal to prevent or reduce fistula-related ischemia by using medications that improve the properties of erythrocytes.
- Carotid-jugular fistula in rats is a feasible model of arteriovenous fistula, especially for studies about fistula-related microcirculatory and systemic changes.
- Carotid-jugular fistula has significant effects on the remote organs, since it results in significant hemodynamic differences, left ventricular hypertrophy and significant alteration of the liver microcirculation.

  The ligation or banding of AVF lead to immediate hemodynamic changes, therefore it should be considered especially in the critical cases.

6. Authenticated list of Candidate´s Publications

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Registry number: DEENK/39/2021.PL
Subject: PhD Publication List

Candidate: Souleiman Ghanem
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Ghanem, S.**, Somogyi, V., Tánczos, B., Szabó, B., Deák, Á., Németh, N.: Modulation of micro-rheological and hematological parameters in the presence of artificial carotid-jugular fistula in rats.
   Clin. Hemorheol. Microcirc. 71 (3), 325-335, 2019.
   DOI: http://dx.doi.org/10.3233/CH-180411
   IF: 1.741
2. **Ghanem, S.**, Tánczos, B., Deák, Á., Bidiga, L., Németh, N.: Carotid-Jugular Fistula Model to Study Systemic Effects and Fistula-Related Microcirculatory Changes.
   J. Vasc. Res. 55 (5), 268-277, 2018.
   DOI: http://dx.doi.org/10.1159/000491930
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