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Improvement of intraoperative methods for the prevention of ischemic-reperfusion injury of the renal graft (experimental and clinical study)

specialty "6D110100 – Medicine"

Dissertation for the degree of Doctor of Philosophy (PhD)

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BACKGROUND

Kidney transplantation is currently the only radical treatment for end-stage kidney diseases that allows the patient to get rid of hemodialysis and its side effects and, thus, significantly improves the quality and duration of life. However, a kidney graft is inevitably subject to ischemia, which always accompanies the surgical process of transplantation, before it is included in the recipient's body's vital activity [1]. These changes in the organ begin with the moment of clamping the renal artery in living donors, and in cadaveric donors - with brain death, and continue with the preservation of the kidneys and during the imposition of vascular anastomoses. Subsequent reperfusion of the organ initiates a complex immune-inflammatory process in the renal tissue, defined as ischemic-reperfusion injury (IRI).

IRI is the primary and main cause of acute kidney injury after surgery, leading to varying degrees of severity of graft function disorders up to its complete loss [2, 3, 4]. Delayed graft function (DGF) caused by IRI is observed in up to 25-47% of cases after all kidney transplants [2]. There is a direct and significant relationship between IRI and DGF, between the severity of IRI and the frequency of acute rejection episodes [5, 6]. Thus, DGF occurs in approximately 25-50% of cases in cadaveric transplants, and up to 17% in living donor transplants [7-12]. The frequency of acute kidney rejection episodes is more often observed in recipients with DGF than in patients without DGF – 49% versus 35% [13]. Moreover, the postoperative development of DGF extends the time of hospitalization and significantly increases the cost of additional treatment for the recipient [14].

The risk of graft loss in combination with mortality in patients with DGF is 2 times higher than in recipients without DGF [15]. Moreover, of all recipients with postoperative DGF, 10% may have a lethal outcome [16].

Currently, there are no accepted unified tactics or clinical recommendations for the prevention and / or treatment of IRI of any transplanted organ. Despite the fact that the kidney is the most studied organ for IRI, there are also no methods that fully meet the need for reducing DGF.

Various approaches are proposed to reduce the negative effects of ischemia and reperfusion during kidney transplantation. This includes both preoperative and intra - and postoperative methods of prevention and treatment. Most of the proposed methods are at the stage of experimental or clinical studies and require expensive maintenance and / or complex procedures.

All available methods, such as dietary preconditioning, various methods of organ storage, various types of machine perfusion, ischemic pre-and postconditioning, the use of mesenchymal stem cells, treatment and prevention with pharmacological drugs, etc. [17] are not universal enough and are not used routinely in clinical practice. Among the listed methods of preventing IRI, pre-and postconditioning is easy to perform and does not require any additional costs. However, their clinical effect is questioned and requires further research [18, 19].

Thus, the problem of kidney transplant IRI and its prevention remain relevant today. It is necessary to analyze and improve existing surgical approaches, with the development of a simple and effective method for the prevention of kidney transplant IRI, which determined the main goal and objectives of this work.

Our Hypothesis:

H (1): retrograde venous reperfusion reduces the renal transplant ischemic-reperfusion injury.

Aim of the study:

To improve the results of kidney transplantation by intraoperative renal graft retrograde venous reperfusion methods for the prevention of ischemic-reperfusion injuries.

Research objectives:

1. To develop an intraoperative method for the prevention of ischemic-reperfusion injury to the renal graft by using retrograde venous reperfusion;
2. To study the effect of retrograde venous reperfusion on ischemic-reperfusion injury of the kidney in an experiment;
3. To evaluate the clinical efficacy of retrograde venous reperfusion of a renal graft during kidney transplantation;
4. To analyze the long-term results of using the intraoperative method of retrograde venous reperfusion of a renal graft in kidney transplant recipients.

MATERIALS AND METHODS

This study included 2 stages: experimental and clinical.

The experimental study was conducted on two bases:

- Animal Research Laboratory of “Başkent” University (Ankara, Turkey); an animal model was developed;

- Cadaveric Center of the “West Kazakhstan Medical University named after M. Ospanov”; approved by the Local Bioethics Committee of the West Kazakhstan Medical University (No. 10 dated 04.12.2021).

Ten male rabbits of the New Zealand white breed with an average weight of 4550 ± 70.7 g were used in the experimental study.

All rabbits were kept in a special vivarium and were on a standard food ration, according to the requirements for the maintenance of experimental animals, in compliance with temperature and light conditions.

The model of bilateral renal ischemia (by Wei Q et al., 2012 and Doulamis IP et al., 2021) was used for the experiment [20, 21]. Laparotomy and isolation of both kidneys and their vessels were performed according to the recommendations of the surgical protocol of animal models [22].

The experimental model with bilateral renal ischemia followed by reperfusion for this experimental study in rabbits was developed in the certified animal laboratory of Başkent University and was approved by the " Ethical Committee for Experimental Animal Research "of Başkent University, Ankara, Turkey (ProjectNo.: E-94603339-604.01.02-38157).

Two groups were formed:

- *group A, the main group* – 10 left kidneys of rabbits, with retrograde venous reperfusion (RVRP) before arterial reperfusion;

- *group B, control group* – 10 right kidneys of rabbits that underwent only antegrade arterial reperfusion without prior retrograde venous reperfusion.

Under intramuscular anesthesia (VetaKetam, Vet-Agro, Lublin, Poland - 60 mg/kg and Vetaxil, Vet-Agro, Lublin, Poland - 10 mg/kg), laparotomy was performed with the isolation of both kidneys, abdominal aorta and inferior vena cava. After 20 minutes of ischemia of kidneys, both kidneys were flushed through a cannula inserted into the abdominal aorta with a cold preserving solution of HTK (Custodiol, Dr. Franz Koehler Chemi GmbH, Germany) at a temperature of 4-5°C. Retrograde reperfusion of the left kidney was performed with venous blood from the inferior vena cava through the left renal vein. After that, a typical arterial reperfusion of both kidneys was initiated.

Forty-eight hours after the operation, the rabbits underwent bilateral nephrectomy under identical anesthesia for histopathological examination.

The histological study was conducted on the basis of the morphological laboratory of the Department of Histology of the “West Kazakhstan State Medical University”, Aktobe, Kazakhstan.

After cutting out the material from the kidneys, its fixation was carried out. A 10% buffered formalin solution was used as a fixative. Further, sections with a thickness of 4-5 microns were stained with hematoxillin-eosin according to the generally accepted method. The material was evaluated using a light microscope at magnification ×50; ×100; ×400. Microscopy of histological preparations was performed using a laboratory medical video microscope "Axio Lab A1", registration certificate-RK-MT-7 No. 009046. Name of the holder of the registration certificate "Carl Zeiss Microscopy GmbH", manufacturer "Carl Zeiss Meditec", Germany, date of state registration: 17.08.2018, №N016546.

In the prepared sections, the degree of damage and/or pathological changes in the renal tissues of the following histological parameters were evaluated:

- Desquamation of the tubular epithelium;
- Tubular debris;
- Vacuolization;
- Interstitial edema;
- Glomerular shrinkage;
- Apoptotic nuclei;
- Brush border loss.

The degree of severity of each of the histopathological parameters in the studied preparations was evaluated from 0 to 3 points, according to the semi-quantitative analysis scale according to Martin Alexander, 2010 [22], where the points meant:

"0" – no damage or changes,

"1" – low degree of damage / changes,

"2" – moderate degree of damage / changes,

"3" – severe damage / changes.

Clinical trial

The research work was carried out at the clinical base of the Department of Surgical Diseases No. 2 with Urology of the "West Kazakhstan Medical University" and "Aktobe Medical Center", Department of Elective Surgery and Transplantation.

The analysis of the materials of the clinical study is based on the results of performing heterotopic kidney transplantation from living donors in 60 patients with end-stage CKD, operated on from 2018 to 2023 at the Aktobe Medical Center.

This clinical study was approved by the Bioethical Commission of the "West Kazakhstan Medical University" (conclusion No. 55A. 02.09.2021) and registered in the International Register of Clinical trials *ClinicalTrials.gov* (ID: NCT05179434). Kidney transplants to recipients of both groups were performed after the approval of the Ethical Commission of the Health Department of Aktobe region. All kidney recipients from the main and control groups were examined in accordance with the clinical protocol dated December 14, 2017 (Protocol No. 35) developed by the Ministry of Health of the Republic of Kazakhstan.

Research design.

Type of study: monocentric clinical trial, with partial historical control.

Number of participants: 60 (with 95% CI and 62% GP, the required sample size is 54).

Research model: longitudinal.

Disguise: none.

Main goal: prevention.

Dates: September 2020 - May 2023.

The patients were divided into 2 groups:

The main group consisted of 30 recipients with retrograde venous reperfusion of a renal graft before a typical arterial one.

The control group consisted of 30 recipients with only typical arterial reperfusion.

Donors from both groups of patients underwent identical laparoscopic nephrectomy. In both study groups, patients received the same three-component immunosuppression regimen after surgery: calcineurin inhibitors (tacrolimus), mycophenolate mofetil and steroid.

Research criteria.

General criteria:

age for research: adult;

gender for research: all (women and men);

availability of volunteers: none;

Blinding: no;

Inclusion criteria:

- patients with end-stage CKD – KDIGO CKD Stage IV-V, 2020 [23] – who are ready for kidney transplantation;
- donors of recipients with laparoscopic kidney removal;
- the level of HLA-antibodies is less than 20% – to exclude the immunological effect of the body on the graft immediately after the transplant surgery;
- informed consent to participate in the study;

Exclusion criteria:

- recipients who are preparing for a combined kidney and other organ transplant;
- recipients with previously performed transplantation of another organ;
- recipients preparing for transplantation with a different immunosuppressive regimen;
- upcoming AB0-incompatible kidney transplantation;
- the level of HLA-antibodies is more than 20%;
- the presence of a history of malignancy;
- mortality of the recipient within one year after transplantation from extrarenal pathologies;
- pregnant women.

To compile the initial clinical characteristics of patients in both groups, the following clinical and laboratory parameters were used:

- age,
- gender,

- relationship to the donor,
- body mass index (BMI),
- duration of hemodialysis up to the moment of transplantation (months),
- baseline creatinine (μmol/L) and urea (mmol/L) blood serum immediately before transplantation,
- estimated GFR according to the accepted formula CKD-EPI before transplantation (ml/min/1.73 m^2),
- the level of HLA-antibodies in the blood before surgery (%),
- duration of primary thermal ischemia of the graft (minutes),
- duration of cold graft ischemia (minutes),
- duration of secondary thermal ischemia of the graft (minutes),
- the total duration of the transplant operation in the recipient, excluding the time of the donor operation (minutes).

Methods of research

Evaluation of immediate results.

Clinical and laboratory parameters:

- Serum urea and creatinine levels – on the 1st, 4th, 7th, and 14th days after transplantation-in both groups.
- Calculation of eGFR – 1st, 4th, 7th, 14th day after transplantation-in both groups. The calculation was performed using an online calculator using a single accepted formula CKD-EPI according to the recommendations of the National Kidney Foundation [24].
- gases analysis of kidney reperfusate and blood from the iliac vein-intraoperatively in patients in the group with RVRP. The composition of retrograde blood flowing from the renal artery was compared with the composition of blood from the central vein (left iliac vein.
- Measurement of daily diuresis – on the 1st, 4th, 7th and 14th days after transplantation – in both groups. Daily diuresis (ml) in the first seven days after organ ischemia and its subsequent reperfusion reflects the severity of IRI of tubular cells expressed as the total volume of hyperfiltration.

Instrumental methods:

- Renal graft ultrasound – on the 1st, 4th, 7th, and 14th days after transplantation – in both groups. The ultrasound machine automatically measures the resistance index (RI) of the renal graft, which is determined by the formula: (peak systolic velocity – end-diastolic velocity) / peak systolic velocity, which expresses the percentage decrease in end-diastolic blood flow in the renal vessels relative to the maximum systolic blood flow.

Evaluation of long-term results.

Clinical and laboratory parameters:

- the level of urea and creatinine in the blood serum - on the 30th, 60th day and 1 year after transplantation-in both groups. A dynamic comparison of creatinine and urea levels in both groups was performed.
- Calculation of GFR – 30th, 60th day and 1 year day after transplantation-in both groups. The calculation was carried out using an online calculator using a single accepted formula CKD-EPI.
- the level of HLA-antibodies in the blood serum-on the 30th day and one year after transplantation-in both groups. A dynamic comparison of the level of antibodies (%) in the blood of recipients of both groups was carried out.

Instrumental methods:

- USG of the renal graft – 30th, 60th day and 1 year after transplantation – in both groups. Measurement of the renal graft parenchymal resistance index (RI), which reflects the degree of renal tissue edema.

Graft survival is the rate of loss of kidney graft function within one year.

Statistical analysis was performed using SPSS version 20.0 for Windows (SPSS Inc, Chicago, IL, 2020). Use the Student's t-test was usedin the samples, and the Fischer test was used for small samples. To compare the nonparametric variables of the two groups with the median values, the Mann-Whitney U-test was used as a two-sample criterion for independent samples. The Xi-square test was used to test the hypothesis of a difference in population distributions or to determine the correlation. Statistical differences between the two groups were considered significant at $p<0.05$.

RESULTS

Results of the experimental study

To confirmя the hypothesis that PVRP reduces the degree of IRI, anexperimental study was conductede исследовани. For this purpose, an animal model with bilateral renal ischemia was used with further RVRP of the left kidney and histological analysis of both kidneys 48 hours after exposure.

Based on the results of histological studies, a comparative assessment of pathomorphological changes in the kidney tissues of both groups was carried out according to the following seven parameters: epithelial desquamation, patency of the tubules, vacuolization, interstitialëedema, glomerular wrinkling, nuclear apoptosis, and loss of the brush border of the proximaltubules.

Results of histological studies with a quantitative assessment ofcpathological changes in the renal tissues of both groups:

1) Desquamation of the epithelium.

In the control group, tubular epithelial desquamation was detected in seven out of ten kidneys. The total degree of epithelial desquamation in all seven kidneys of the control group was 15 points (average 1.50±1.18 points). At the same time, in the main group, desquamation was observed only in five out of ten renal tissue samples (on average, 0.70±0.82 points). Moreover, in this group, changes in three of the five kidneys were estimated at 1 point – a slight degree of epithelial desquamation. Thus, in the renal tissues of the main group, epithelial desquamation was observed 1.4 times less frequently than in the controlgroup, and in most cases it was mild, p=0.04

2) Tubular debris.

In the control group of ten kidneys, the patency of the tubules was impaired in nine, and in the main group – in five out of ten.Moreover, in two kidneys, changes were mild (22.2%), in five – moderate (55.6%), in two kidneys – severe (22.2%) obstruction of the renal tubules. The degree of these pathological changes in all ten kidneys in the control group averaged 1.80 ± 0.92 points, compared to 0.60±0.70 points in the main group, p=0.002. That is, in the main group, the degree of tubule blockage after retrograde renal reperfusion was less pronounced compared to the control group.

3) Vacuolization.

Pathological changes in renal tissues in the form of vacuolization of cells were present in nine kidneys out of ten in each of the groups – 90%. In the main group, the severity of vacuolization averaged 0.50±0.97 points compared to 1.90±0.99 in the control group. The severity of these changes in both groups was statistically the same, p=0.18.

4) Interstitial edema.

Interstitial edema was observed in all 20 kidneys studied. Although in the main group, the total total degree of edema was lower, than in the control group (14 and 18 points, respectively). Statistically, the degree of interstitial edema was the same in both groups and amounted to 1.40 ± 0.52 points in the main group and 1.80 ± 0.79 points in the control group, p=0.09.

5) Glomerular shirinkage.

Glomerular shirinkage was found in nine out of ten kidneys in the control group, and one of them had severe changes (Figure 18). While in the main group, shirinkage was detected only in four samples, and all four were slightly pronounced. On average, the degree of glomerular shirinkage was 0.40 ± 0.52 points in the main group and 1.70±0.82 points in the control group. Thus, in the histological samples of the main group using the RVP technique, pathological changes in the form of glomerular shirinkage were significantly less pronounced, p=0.0002.

6) Nuclear apoptosis.

In the control group, nuclear apoptosis was detected in the tissues of eight out of ten kidneys: in two kidneys, changes were mild, in three – moderate and in three – severe. In the main group, only four tissue samples showed mild apoptosis. Thus, the total degree of nuclear apoptosis in the control group averaged 1.70±1.16 points, and in the main group – 0.40±0.52 points. When comparing the data in the control group, pronounced apoptosis of cell nuclei was observed compared to the group with RVRP, p=0.002.

7) Brush border loss.

Brush border loss of the tubules was observed in eight cases out of ten kidneys in the control group. The severity of these changes was 1.30±0.95 points, and a total of 13 points. In this group, Brush border loss was mild in three kidneys, moderate in three, and severe in one case. In the main group, this

change was found in five samples, but they were slightly pronounced. Brush border loss of the proximal tubules in the main group averaged 0.50±0.53 points, a total of 5 points. Thus, the group with RVRP showed minimal damage to the Brush border loss of the tubules compared to the control group, p=0.01.

A comparative analysis of histological changes in kidney tissue revealed a statistically significant difference in five out of seven indicators in the main group. Epithelial desquamation, tubular debris, glomerular shirinkage, nuclear apoptosis, and brush border loss were less pronounced in the renal tissue samples in the group with RVRP (p<0.05). At the same time, there were no significant differences in the degree of cell vacuolation and interstitial edema in samples from renal tissues of both groups (p>0.05).

Thus, the analysis of the obtained pathomorphological data showed that previous renal RVRP can significantly reduce the severity of pathological changes observed after ischemia and renal reperfusion. Moreover, in the main group, compared with the control group, the severity of epithelial desquamation was 2.1 times lower, tubular debris – 3 times, glomerular shirinkage and nuclear apoptosis – 4.3 times, and brush border loss – 2.6 times (p<0.05).

Results of the clinical trial

The results of the experimental study showed that the method of RVRP of the kidney significantly reduces its IRI and served as a justification for using this method in a clinical setting.

The clinical study included 60 patients with a diagnosis of stage 4-5 CKD, who underwent heterotopic kidney transplantation from living donors. The main group consisted of 30 patients who underwent renal graft RVRP before antegrade arterial reperfusion. The control group included 30 patients with typical antegrade arterial reperfusion.

Indications for kidney transplantation in all recipients were CKD stage 4-5 with GFR <30 ml/ min/1.73m^2 according to the KDIGO, 2020 criteria [25]. All 60 recipients received kidney transplants from living related donors. All 60 donors in both groups had their kidneys removed laparoscopically to ensure uniformity of the study. The majority of donors in both groups for their recipients were first-generation relatives (91.7%) – sisters or brothers, mother or father, and children.

All recipients were examined according to Health Ministry clinical protocols prior to surgery. Patients in both groups received a hemodialysis session the day before the transplant.

In order to confirm the presence of ischemic damage to the transplanted kidney, blood was collected from the recipient's iliac vein for gas analysis immediately before the start of RVRP. Next, retrograde reperfusion was performed according to the proposed method. Retrograde blood in the volume of 80-100 ml flowed out of the left "window" of the arterial anastomosis. This retrograde blood flowing out of the transplanted kidney was collected for analysis of gas composition.

A comparative analysis of the gas composition of retrograde blood with blood samples taken from the iliac vein of recipients of the main group immediately before RVRP revealed that the pH in retrograde blood was lower than in blood from the iliac vein – 6.40±0.39 and 7.38±0.03, respectively (p<0.01). This confirms the presence of an excess of bases (BEecf) in the blood flowing from the kidney compared to the blood from the recipient's central vein: -25.86±2.49 mmol/L and -1.98±0.21 mmol/L, respectively (p<0.01). High levels of lactate and potassium were also found in retrograde blood: 5.2±0.55 mmol/L and 13.13±1.77 mmol/L versus 1.08±0.38 mmol/l and 4.30±0.36 mmol/L in the blood from the iliac vein (p<0.01). And the level of calcium was significantly lower in retrograde blood (0.54±0.06 mmol/L) than in the blood from the iliac vein (1.13±0.05 mmol/L) of recipients (p<0.01).

The data obtained convincingly showed the presence of deep metabolic disorders in kidney tissues due to the period of cold and warm ischemia and subsequent reperfusion. Deep acidosis, a pronounced excess of BEecf, a high concentration of lactate and potassium, and a low content of calcium in retrograde blood confirmed the development of IRI in the tissues of the transplanted kidney.

Postoperative characteristics.

On the first day after surgery, the recipients of the main group had a diuresis of 4979.7±1781.9 ml/dayl, and in the control group: 7943.3±2391.6 ml/day. In the following days after surgery (days 4, 7, and 14), pronounced polyuria occurred in comparison with the initial diuresis indicators in the main group (p<0.01). So, on day 4, the daily diuresis was 3966.7 ± 723.9 ml/day in the group with RVRP and 4526.7±661.6 ml/day in the control group (p<0.01). On day 7, polyuria also persisted in the control group and amounted to 3441.7±448.4 ml/day compared to 3048.3±394.1 ml/day in the main group (p<0.01). It should be noted that on day 14, minor polyuria still persists in the control group

(2361.7±286.4 ml/day), while in the main group there is an earlier normalization of daily diuresis ($p<$ 0.02).

This fact reflects the fact that IRI of a kidney transplant was more pronounced in the control group than in the main group. in the control group, polyuria persisted and was significantly higher than the daily diuresis in the main group.

The recipients' serum creatinine levels on the 1st, 4th, 7th, and 14th days after surgery were significantly lower in the main group ($p<0.05$), compared with those in the group without RVRP. The dynamics (trend) of the decrease in creatinine levels was as follows: Day 1 – 264.4±78.4 vs 299.0±42.1 μmol/L; Day 4 – 91.0±12.3 vs 101.7±6.0 μmol/L; Day 7 – 78.2±14.3 vs 89.8±10.4 μmol/L; Day 14 – 76.8±10.5 vs. 89.1±11.4 μmol/L, respectively. At the same time, in the main group with the use of the RVP method, normalization of creatinine levels in patients is observed on the 4th day, while in the control group, a tendency to normalization was noted only from the 7th day after transplantation. A similar pattern is observed in the urea level.

The eGFR levels on days 1, 4, 7, and 14 after surgery were significantly higher in the main group ($p<0.05$) than in the control group. Dynamics of eGFR increase in the comparative aspect: day 1 – 26.7±12.6 vs. 22.1±6.4 ml/min/1.73m^2; Day 4 – 90.2±18.8 vs. 80.3±15.9 ml/min/1.73m^2; Day 7 – 104.9±17.2 vs. 92.1±21.8 ml/min/1.73m^2 and day 14 – 103.5±17.5 versus 95.4±12.9 ml/min/1.73m^2, respectively. As can be seen from the results obtained, in the group of patients with RVRP, eGFR indicators starting from 4 days after surgery tend to improve the filtration capacity of kidney transplants.

When performing US of the kidney grafts, the resistance index was: day 1 – 0.53±0.06 and 0.73±0.11; day 4 – 0.66±0.14 and 0.70±0.08; day 7 – 0.64±0.09 and 0.68±0.07; day 14 – 0.54±0.04 and 0.66±0.06 in the main and control groups, respectively. In the two groups, there were no statistically significant differences in RI indicators ($p>0.05$).

The average duration of postoperative hospital stay of recipients in the main group was 17.9±2.2 days, and in the control group: 24.3±3.13 days.

In the main group of patients using the PVRP method, the average level of creatinine and urea on the 30th, 60th days and one year after transplantation remained within the normal range, while in the control group this indicator still remained elevated. Creatinine values in the group with RVRP were: at the 30th Day – 69.7±10.8 vs. 86.2±4.7 μmol/L; at the 60th – 79.9±8.03 vs. 92.03±8.1 μmol/L; at 1 year – 85.4±7.9 vs. 91.43±8.9 μmol/L, respectively ($p>0.05$).

The eGFR levels on days 30, 60, and one year after surgery were significantly higher in the main group ($p<0.05$) compared to the control group without RVRP. The average eGFR level one month after transplantation in the control group was 100.7±10.7 ml/min/1.72m^2, in the main group – 112.9±13.8 ml / min/1.72m^2; on day 60: 92.2±14.6 and 106.3±14.5; after 1 year – 92.3±16.2 and 99.5±13.9 ml/ min/1.72m^2, respectively. As can be seen from the results obtained, in the group of patients with RVRP, eGFR indicators in the long-term period after surgery remain at high levels, which indicates the optimal filtration function of renal grafts.

When conducting an immunological analysis one month after transplantation, no HLA-antibodies of both classes I and II were detected in the blood of recipients of the main group. While in the recipients of the control group on the 30th day after surgery, the average level of class I HLA-antibodies was 1.8±4.6%, and class II – 0.83±2.7% ($p<0.01$). One year after transplantation, the blood level of class I HLA-antibodies was not detected in the recipients of the main group, while class II antibodies were 0.2±1.1%. And in the recipients of the control group, after 1 year, the level of class I HLA-antibodies averaged 2.1±4.9%, and class II – 1.9±4.5% ($p<0.01$).

Graft survival.

The analysis of long-term results revealed that within one year, the loss of kidney transplant function in the control group was observed in 4 recipients, which was 13.3,%, in the main group this complication was only in 1 patient – 3.3% ($p=0.16$).

In the control group, the causes of graft loss were:

- acute sever respiratory infection – 1;
- rejection – 3.

Graft loss in one recipient in the main group occurred against the background of extrarenal pathology 8 months after surgery. A severe acute respiratory infection caused graft dysfunction, followed by a complete loss of its function. While in the control group, the main cause of graft loss

(75%) was renal pathology in the form of chronic kidney rejection. Thus, the overall one-year survival of kidney transplants in both groups did not differ statistically. When analyzing the survival rate of a kidney transplant without taking into account cases of loss of kidney function due to extrarenal pathologies, according to the Gehan-Breslow-Wilcoxon test, a statistically significant difference was found between the survival rates of the main and control groups (p=0.04). The hazard ratio for treatment in control group (without RVRP) was higher than in main group with RVRP (95% CI: 1.099 to 58.37), as calculated using the Mantel-Haenszel method.

Thus, retrograde venous reperfusion of renal transplants, which precedes antegrade arterial reperfusion, reduces the effects of IRP of the renal parenchyma after its ischemia and reperfusion, and accelerates the normalization of renal functional parameters. This effect of RVRP increases its survival rate and reduces the risk of graft loss within one year directly from renal causes.

Based on the results of the study, the following **conclusions can be drawn:**

1. Retrograde venous kidney reperfusion in recipients is an effective intraoperative method for preventing ischemic-reperfusion injury to the graft. It is technically simple to perform and does not require any additional tools or equipment.

2. Результатами As a result of an experimental study in animal models, it was found that preliminary retrograde venous reperfusion of an ischemic kidney significantly reduces the severity of ischemic-reperfusion damage compared to typical antegrade arterial reperfusion: epithelial desquamation by 2.1 times, tubular detachment and obstruction by 3 times, glomerular wrinkling and nuclear apoptosis by 4.3 times, and damage to the brush border – by 2.6 times.

3. The intraoperative method of prevention of ischemic-reperfusion injury of the renal graft by retrograde venous reperfusion allows to accelerate the normalization of functional parameters of the kidney by 2 times, to reduce the time of postoperative stay of patients in the hospital by 1.4 times.

4. In the long-term postoperative period, the method of retrograde venous reperfusion of a renal graft allows maintaining the optimal filtration function of the transplanted kidney, increasing its survival and reducing the renal graft loss within one year by 1.2 times.

Practical recommendations

1. During kidney transplantation, intraoperative retrograde venous reperfusion of the graft should be performed to prevent ischemic reperfusion injuryуретроградную венозную реперфузию .

2. After the venous anastomosis is completed, an arterial anastomosis should be applied without tightening the suture, leaving a lumen for the outflow of retrograde blood.

3. Before a typical arterial reperfusion, retrograde blood flow through the renal vein should be performed so that venous blood fills the graft and flows out through the renal artery in a volume of 80-100 ml. Next, the suture of the arterial anastomosis should be tightened and bandaged.

4. After retrograde venous reperfusion, perform antegrade arterial reperfusion of the graft through the renal artery.

Scientific novelty

1) A new intraoperative method for the prevention of ischemic-reperfusion injury to the renal graft was developed by using retrograde venous kidney reperfusion (Patent for the invention of the Republic of Kazakhstan No. 2021/0443.1 - 27.01.2021);

2) In the experiment, the positive effect of the retrograde venous reperfusion method on ischemic-reperfusion kidney damage was established;

3) The clinical efficacy of retrograde venous reperfusion of a renal graft during kidney transplantation was evaluated;

4) Based on the study of the long-term results of using the intraoperative method of retrograde venous reperfusion of a renal graft in recipients, its high effectiveness in kidney transplantation was revealed.

Practical significance

The developed intraoperative method for the prevention of ischemic-reperfusion injury to the renal graft by using retrograde venous reperfusion in recipients can improve the results of kidney transplantation.

Implementation forms

1. Patent for the invention of the Republic of Kazakhstan No. 2021/0443.1 - 27.01.2021 "Prevention of ischemic-reperfusion damage to the renal graft by using retrograde venous kidney reperfusion";

2. Practical Healthcare Implementation Act No.283 of 19.12.2022: "Method of renal graft retrograde venous reperfusion";

3. Implementation Act No.16 of 01.02.2023 in practical healthcare: "Method of local intra-ureteral cooling of a kidney graft during implantation in a recipient".

Performances:

1) XXXIX Congress of the TTS-2024 (Turkey, Istanbul, 2024 - September);

2) “Asian Transplantation Week 2023” (Republic of Korea, Seoul, 2023 - November);

3) III Congress of Surgeons of Kazakhstan with international participation (Kazakhstan, Almaty, 2022 - September);

4) XXX Congress of the TTS-2022 (Argentina, Buenos Aires, 2022 - September);

5) “Asian Transplantation Week 2021” (Republic of Korea, Seoul, 2021 - October);

6) International Scientific and Practical Forum of the Caspian Littoral Countries "Technologies and Innovations" (Astrakhan, Russia, 2021 - March);

7) “Asian Transplantation Week 2020” (Republic of Korea, Seoul, 2020 - December).

Publications 4 articles: Q2 (68%) - 1, Q3 (38%) - 1, Q4 (24%) - 2:

1. Rysmakhanov, M., Smagulov, A., Sultangereyev, Y. et al. *Evaluation of the effect of retrograde venous renal reperfusion in rabbits on ischemic reperfusion injury: an experimental study*. Comp Clin Pathol (2024). https://doi.org/10.1007/s00580-024-03606-1.

2. Rysmakhanov MS, Zare A, Smagulov AS, Abenova NA, Mussin NM, Sultangereyev YB, Zhakiyev BS, Kuttymuratov GK, Haberal M et all. *Comprehensive Overview of Innovative Strategies in Preventing Renal Ischemia-reperfusion Injury: Insights from Bibliometric and In Silico Analyses*. Curr Pharm Des. 2024 Apr 26. doi: 10.2174/0113816128283420240409050754.

3. Rysmakhanov M, Yelemessov A, Mussin N, Yessenbayev D, Saparbayev S, Zhakiyev B, Sultangereyev Y. *Two- and three-dimensional laparoscopic donor nephrectomy: a comparative study of a single-center experience*. Korean Journal of Transplantation 2022;36:104-110. https://doi.org/10.4285/kjt.22.0003.

4. Rysmakhanov M, Smagulov A, Mussin N, Kaliyev A, Zhakiyev B, Sultangereyev Y, Kuttymuratov G. *Retrograde reperfusion of renal grafts to reduce ischemic-reperfusion injury*. Korean Journal of Transplantation 2022;36:253-258.https://doi.org/10.4285/kjt.22.0053.

Abstracts 7: Q1 (96%) - 2, Q4 (24%) – 4, KKSON-1:

1. Rysmakhanov M, Kuttymuratov G. *Possibility of using retrograde reperfusion renal graft to reduce ischemic-reperfusion injury*. Korean Journal of Transplantation 2020;34:108-108. https://doi.org/10.4285/ATW2020.PO-1051.

2. Rysmakhanov M, Kuttymuratov G. *Retrograde reperfusion of renal graft to reduce ischemia-reperfusion injury*. Korean Journal of Transplantation 2021;35:87-87. https://doi.org/10.4285/ATW2021.OP-1110.

3. Rysmakhanov, Myltykbay; Kuttymuratov, Gany; Mussin, Nadiar. P2.17: *Retrograde Reperfusion of the Renal Graft in Adult Recipient To Reduce Ischemia-Reperfusion Injury*. Transplantation 106(9S):p S513, September 2022. | DOI: 10.1097/01.tp.0000888216.53264.cc

4. Rysmakhanov M. S., Musin N. M., Zhakiev B. S., Haberal M. *Retrograde venous reperfusion of the kidney-an experimental study*. Bulletin of Surgery of Kazakhstan 2022(C1); p. 152.

5. Rysmakhanov M, Karakaya E, Akdur A, Haberal M. *The effect of retrograde venous renal reperfusion on ischemia-reperfusion injury in rabbits*. Korean Journal of Transplantation 2022;36:31-31. https://doi.org/10.4285/ATW2022.F-1279.

6. Mussin N, Rysmakhanov M, Sultangereyev Y. *Three-dimensional laparoscopic donor nephrectomy: single-center experience*. Korean Journal of Transplantation 2022;36:214-214.https://doi.org/10.4285/ATW2022.F-3530.

7. Sultangereiyev, Yerlan; Rysmakhanov, Myltykbay; Zhakiyev, Bazylbek; Mussin, Nadiar; Haberal, Mehmet. P.289: *Retrograde venous reperfusion of the kidney – An experimental study*. Transplantation 108(9S): September 2024. | DOI: 10.1097/01.tp.0001067952.64313.08.

Personal contribution of the author

The doctoral candidate personally conducted the experimental work and its analysis. Directly by the author himself, patients were selected for study groups, clinical examinations of participants were performed, all medical documentation was kept, an electronic database was formed, primary statistical processing and analysis of the obtained research results were performed. The applicant has developed and obtained a patent for an invention of the Republic of Kazakhstan ("Kazpatent") No. 2021/0443.1. The author was directly involved in all surgical procedures for patients, supervised after the operation, and examined them in the immediate and long-term postoperative periods. The applicant independently prepared all munuscripts and reports, wrote a dissertation.

REFERENCES

1) Qiu L, Zhang ZJ. Therapeutic Strategies of Kidney Transplant Ischemia Reperfusion Injury: Insight From Mouse Models. Biomed J Sci Tech Res. 2019;14(5):002617. Epub 2019 Feb 20. PMID: 31093605; PMCID: PMC6513342.

2) Cavaille-Coll, M., Bala, S., Velidedeoglu, E., Hernandez, A., Archdeacon, P., Gonzalez, G., Neuland, C., Meyer, J. Albrecht, R. (2013). Summary of FDA workshop on ischemia reperfusion injury in kidney transplantation. Am J Transplant, 13(5), 1134-1148. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23566221. doi:10.1111/ajt.12210

3) Pefanis A, Ierino FL, Murphy JM, Cowan PJ. Regulated necrosis in kidney ischemia-reperfusion injury. Kidney Int. 2019 Aug; 96(2):291-301. doi: 10.1016/j.kint.2019.02.009. Epub 2019 Mar 7. PMID: 31005270.

4) Jang HR, Rabb H. The innate immune response in ischemic acute kidney injury. Clin Immunol. 2009 Jan;130(1):41-50. doi: 10.1016/j.clim.2008.08.016. Epub 2008 Oct 14. PMID: 18922742; PMCID: PMC2646108.

5) Yarlagadda SG, Klein CL, Jani A. Long-term renal outcomes after delayed graft function. Adv Chronic Kidney Dis. 2008 Jul;15(3):248-56. doi: 10.1053/j.ackd.2008.04.005. PMID: 18565476.

6) Zhao H, Alam A, Soo AP, George AJT, Ma D. Ischemia-Reperfusion Injury Reduces Long Term Renal Graft Survival: Mechanism and Beyond. EBio Medicine. 2018 Feb;28:31-42. doi: 10.1016/j.ebiom.2018.01.025. Epub 2018 Feb 2. PMID: 29398595; PMCID: PMC5835570.

7) Redfield RR, Scalea JR, Zens TJ, Muth B, Kaufman DB, Djamali A, Astor BC, Mohamed M. Predictors and outcomes of delayed graft function after living-donor kidney transplantation. Transpl Int. 2016 Jan;29(1):81-7. doi: 10.1111/tri.12696. Epub 2015 Oct 26. PMID: 26432507.

8) Irish WD, Ilsley JN, Schnitzler MA, Feng S, Brennan DC. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. Am J Transplant. 2010 Oct;10(10):2279-86. doi: 10.1111/j.1600-6143.2010.03179.x. PMID: 20883559.

9) Chen R, Wang H, Song L, Hou J, Peng J, Dai H, Peng L. Predictors and one-year outcomes of patients with delayed graft function after deceased donor kidney transplantation. BMC Nephrol. 2020 Dec 4;21(1):526. doi: 10.1186/s12882-020-02181-1. PMID: 33276737; PMCID: PMC7716446.

10) Legendre C, Canaud G, Martinez F. Factors influencing long-term outcome after kidney transplantation. Transpl Int. 2014 Jan;27(1):19-27. doi: 10.1111/tri.12217. Epub 2013 Nov 8. PMID: 24138291.

11) Potluri VS, Parikh CR, Hall IE, Ficek J, Doshi MD, Butrymowicz I, Weng FL, Schröppel B, Thiessen-Philbrook H, Reese PP. Validating Early Post-Transplant Outcomes Reported for Recipients of Deceased Donor Kidney Transplants. Clin J Am Soc Nephrol. 2016 Feb 5;11(2):324-31. doi: 10.2215/CJN.06950615. Epub 2015 Dec 14. PMID: 26668026; PMCID: PMC4741043.

12) Al Otaibi T, Ahmadpoor P, Allawi AA, Habhab WT, Khatami MR, Nafar M, Glotz D. Delayed Graft Function in Living-Donor Kidney Transplant: A Middle Eastern Perspective. Exp Clin Transplant. 2016 Feb;14(1):1-11. PMID: 26862818

13) Siedlecki A, Irish W, Brennan DC. Delayed graft function in the kidney transplant. Am J Transplant. 2011 Nov;11(11):2279-96. doi: 10.1111/j.1600-6143.2011.03754.x. Epub 2011 Sep 19. PMID: 21929642; PMCID: PMC3280444.

14) Serrano OK, Vock DM, Chinnakotla S, Dunn TB, Kandaswamy R, Pruett TL, Feldman R, Matas AJ, Finger EB. The Relationships Between Cold Ischemia Time, Kidney Transplant Length of Stay, and Transplant-related Costs. Transplantation. 2019 Feb;103(2):401-411. doi: 10.1097/TP.0000000000002309. PMID: 29863580.

15) Khalkhali HR, Ghafari A, Hajizadeh E, Kazemnejad A. Risk factors of long-term graft loss in renal transplant recipients with chronic allograft dysfunction. Exp Clin Transplant. 2010 Dec;8(4):277-82. PMID: 21143092.

16) Lim WH, Johnson DW, Teixeira-Pinto A, Wong G. Association Between Duration of Delayed Graft Function, Acute Rejection, and Allograft Outcome After Deceased Donor Kidney Transplantation. Transplantation. 2019 Feb;103(2):412-419. doi: 10.1097/TP.0000000000002275. PMID: 29762458.

17) Saat TC, van den Akker EK, IJzermans JN, Dor FJ, de Bruin RW. Improving the outcome of kidney transplantation by ameliorating renal ischemia reperfusion injury: lost in translation? J Transl Med. 2016 Jan 20;14:20. doi: 10.1186/s12967-016-0767-2. PMID: 26791565; PMCID: PMC4721068.

18) Menting TP, Wever KE, Ozdemir-van Brunschot DM, Van der Vliet DJ, Rovers MM, Warle MC. Ischaemic preconditioning for the reduction of renal ischaemia reperfusion injury. Cochrane Database Syst Rev. 2017 Mar 4;3(3):CD010777. doi: 10.1002/14651858.CD010777.pub2. PMID: 28258686; PMCID: PMC6464274.

19) Cheungpasitporn W, Khoury NJ, Thongprayoon C, Craici IM. Is Remote Ischemic Conditioning of Benefit to Patients Undergoing Kidney Transplantation? J Invest Surg. 2019 Jan;32(1):72-74. doi: 10.1080/08941939.2017.1380090. Epub 2017 Oct 12. PMID: 29023177.

20) Wei Q, Dong Z. Mouse model of ischemic acute kidney injury: technical notes and tricks. Am J Physiol Renal Physiol. 2012;303(11):F1487-F1494. doi:10.1152/ajprenal.00352.2012.

21) Doulamis IP, Guariento A, Saeed MY, et al. A Large Animal Model for Acute Kidney Injury by Temporary Bilateral Renal Artery Occlusion. JVisExp. 2021;(168):10.3791/62230. Published 2021 Feb 2. doi:10.3791/62230.

22) Hesketh EE, Czopek A, Clay M, et al. Renal ischaemia reperfusion injury: a mouse model of injury and regeneration. JVisExp. 2014;(88):51816. Published 2014 Jun 7. doi:10.3791/51816.

23) Schick MA, Isbary TJ, Schlegel N, Brugger J, Waschke J, Muellenbach R, Roewer N, Wunder C. The impact of crystalloid and colloid infusion on the kidney in rodent sepsis. Intensive Care Med. 2010 Mar;36(3):541-8. doi: 10.1007/s00134-009-1704-0. Epub 2009 Nov 5. PMID: 19890625.

24) Chadban SJ, Ahn C, Axelrod DA, Foster BJ, Kasiske BL, Kher V, Kumar D, Oberbauer R, Pascual J, Pilmore HL, Rodrigue JR, Segev DL, Sheerin NS, Tinckam KJ, Wong G, Knoll GA. KDIGO Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation. Transplantation. 2020 Apr;104(4S1 Suppl 1):S11-S103. doi: 10.1097/TP.0000000000003136. PMID: 32301874.

25) Shaffi K, Uhlig K, Perrone RD, Ruthazer R, Rule A, Lieske JC, Navis G, Poggio ED, Inker LA, Levey AS. Performance of creatinine-based GFR estimating equations in solid-organ transplant recipients. Am J Kidney Dis. 2014 Jun;63(6):1007-18. doi: 10.1053/j.ajkd.2014.01.436. Epub 2014 Apr 2. PMID: 24703720; PMCID: PMC4113340.