Clinical significance of the abnormal Doppler spectrum of renal blood flow in patients with long-term transplant dysfunction

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Relevance. One of the main causes of graft loss in a recipient after kidney transplantation is acute rejection.

The purpose of the work – to study the Doppler ultrasound measurements of renal transplant blood flow in patients with efficient depuration function and renal graft dysfunction in the late postoperative period following KT.

Materials and methods. As an initial step, an ultrasound examination of renal transplants was performed in 26 patients with creatinine levels within the normative values (group 1) during the period 2014–2015. The second study was based on the ultrasound examination results of the renal transplants in 26 patients with creatinine levels exceeding the standard values (group 2) during the period 2015–2016.

Results. The first group of recipients included 15 male (57.7 %) and 11 (42.3 %) female (the mean age was 31.40 ± 1.67 years). A living related kidney transplantation (LRKT) was performed in 61.54 % of patients, in 38.46 % – a cadaveric kidney transplantation (CKT). In all the patients, plasma creatinine level was within normative values or not exceeding them more than 25 %, on average, ranging from 94 to 130 µmol/L, and its mean value was 114.5 ± 3.85 µmol/L. The second group of recipients consisted of 14 (53.84%) male and 12 (46.16%) female (the mean age was 38.99 ± 2.32 years). LRKT was performed in 6 patients, and CKT in 20 patients. In all the patients plasma creatinine level was above the normal range, on average, ranging from 155 to 629 µmol/L and its mean value was 259.46 ± 35.33 µmol/L.

Conclusions. The obtained data reliably indicate that if TAMX of interlobar arteries less than 15 cm/s, the probability to reveal the clinical signs of renal allograft dysfunction is more than 90 %, regardless of the renal segment evaluation in recipients in the long-term period after organ transplantation.
Relevance

In comparison with transplantation of other organs, such line as kidney transplantation (KT) has a history stretching back more than half a century. For this period, tremendous experience concerning surgical techniques modernization, organ preservation, improvement and optimization of immunosuppression protocols and postoperative management of patients has been accumulated and current survival rates of recipients and renal grafts have been achieved. On the contrary, the advances in KT have led to the fact that a waiting lists for the operation are steadily increasing around the world [1].

In addition to low indicators of quality of life, dialysis patients have much shorter life expectancies. Thus, patients receiving hemodialysis at the age of 40–59 years, live on average 11 years less compared to those who underwent KT. For patients whose age group is 20–39 years, this difference already reaches 17 years [2].

KT today is the method of choice in the treatment of patients with end-stage chronic renal failure, which can significantly improve the quality of life, contributes to a more complete social rehabilitation in this category of patients. One of the main causes of graft loss in a recipient after kidney transplantation is acute rejection. The number and severity of acute rejection cases directly correlate with loss of active nephrons and the duration of graft survival [3].

It is known that the half-life of cadaveric grafts, according to different authors, varies between 8–10 and 10–14 years, depending on the degree of compatibility and quality of organs. For kidneys obtained from living related donors, this figure is 17 years with compatibility beyond the haplotype and exceeds 30 years in main histocompatibility complex antigen-identical donor and recipient [4].

Rejection is defined as general inflammatory process of the graft, caused by a specific immune response of the recipient to the donor’s transplant antigens [5]. Almost all mechanisms of cellular and humoral responses are involved in the interaction between the recipient’s immune system and the antigens of the donor kidney transplant. The interaction between the antigen-specific T-cell receptor and allogeneic HLA antigens is the central event of this reaction. The main manifestation of the immunological conflict is the loss of excretory function of the donor organ or the lack of regeneration [6].

Diagnosis of acute rejection is based on clinical and instrumental studies, laboratory and immunological parameters. At the same time, punch biopsy remains the “gold standard” for assessing the pathological states in transplant nephropathy. Although it is known that punch biopsy can lead to a number of complications such as hematoma, renal infarction, urinoma, etc. [7].

Ultrasound examination is often the primary diagnostic method, which is characterized by non-invasiveness, relative cheapness, imaging at patient’s bed-side, there is no need for intravenous contrast, and it allows rapid and accurate detecting a number of common complications [8].

The purpose of the work

To study the Doppler ultrasound measurements of renal transplant blood flow in patients with efficient depuration function and renal graft dysfunction in the late postoperative period following KT.

Materials and methods

An ultrasound examination of renal transplants was performed in 26 patients with creatinine levels within the normative values (group 1) during the period 2014–2015. This group of recipients included 15 male (57.7 %) and 11 female, mean age was 31.40 ± 1.67 years. Living related kidney transplantation (LRKT) was performed in 61.54% of patients, in 38.46 % – a cadaveric kidney transplantation (CKT). In all the patients, plasma creatinine level was within normative values or not exceeding them more than 25 %, on average, ranging from 94 to 130 µmol/L and its mean value was 114.50 ± 3.85 µmol/L.

The second study was based on the ultrasound examination results of the renal transplants in 26 patients with creatinine levels exceeding the standard values (group 2) during the period 2015–2016. The second group of recipients consisted of 14 male (53.84 %) and 12 female, the mean age was 38.99 ± 3.22 years. LRKT was performed in 6 patients, and CKT in 20 patients. In all the patients plasma creatinine level was above the normal range, on average, ranging from 155 to 629 µmol/L and its mean value was 259.46 ± 35.33 µmol/L.
The ultrasound examination was performed with a Toshiba (Xario) device using a convex multi-frequency transducer at 3–5 MHz and included the state assessment and topometry of kidney transplant, the state of perirenal space assessment, color Doppler and spectral Doppler. To assess the kidney transplant state, we determined the kidney contour, homogeneity and echogenicity of the parenchyma, the clarity of cortical-medullary differentiation, the renal pelvis system and ureter state. Length and front-rear sizes of kidney transplant were dimensioned by topometry. The parenchyma and cortical layer thickness was calculated as the average value of the three measurements in the middle segment of kidney transplant.

The renal arteries blood flow was measured at the renal artery trunk and the interlobar branches of the renal artery (IBRA) levels by the spectral Doppler ultrasound evaluating the linear velocity indices. In addition, “almost angle-independent” indicators of peripheral vascular resistance (resistivity) were used. The renal vessels vascular resistivity was calculated by processing the Doppler waveforms using standard formulas.

The Doppler spectral waveform shape was evaluated and the following parameters were determined: peak systolic velocity (PSV), end-diastolic velocity (EDV), resistance index (RI) and pulsatility index (PI) of the blood flow, systolic-diastolic ratio (SDR), acceleration time (AT), time-averaged maximum velocity (TAMX) both in the renal and interlobar arteries. The angle of insonation was in the range from 30° to 60°. The average indicators of blood flow were analyzed throughout 3–6 cardiac cycles by Dopple spectrum. In addition, the linear blood flow velocity (LFV) in the renal vein was also evaluated.

Serum creatinine concentration was measured using the Jaffe color reaction (Popper method). All biochemical studies were performed at the Central Clinical and Express Biochemical Laboratories of the Zaporizhzhia Regional Clinical Hospital.

The data were first examined for normality using the Kolmogorov-Smirnov test. Parametric and non-parametric methods of data analysis were used. The results were presented as mean and standard error of representativeness of the sample standard value. The study results were processed using the statistical package of the licensed program Statistica® for Windows 6.0 (StatSoft Inc., NAXXR712D833214FAN5), SPSS 17.0 and Microsoft Excel 2010. Separate statistical procedures and algorithms were implemented as specially written macros in the corresponding programs. Statistical significance was defined as P < 0.05 for all types of differences.

**Results**

For the kidney transplant evaluation in patients with normal plasma creatinine levels (group 1), the following results were obtained by B-mode imaging and topometry: parenchyma thickness was 17.23 ± 0.35 mm, cortical substance – 7.15 ± 0.20 mm, cortical-medullary differentiation were defined as clear in the vast majority – 92.31 %, fuzzy – in 7.69 % of the examined patients, the echogenicity of kidney transplant parenchyma was isoechoic in all the patients (100 %). LFV in the renal vein averaged about 34.06 ± 3.10 (27.69–40.43) cm/s. For the renal transplants evaluation in patients with plasma creatinine levels exceeding standard values (with impaired depuration function, group 2), the following results were obtained by B-mode imaging and topometry: parenchyma thickness was 17.35 ± 0.46 (16.46–18, 25) mm, cortical substance – 7.8 ± 0.28 (7.25–8.35) mm. The cortical-medullary differentiation was defined as clear in the majority of the examined patients – in 73.01 %; the echogenicity of parenchyma was increased in 23.1 % of patients. LFV in the renal vein averaged about 23.98 ± 2.22 (19.54–28.43) cm/s.

The results of spectral Doppler indicators of renal graft hemodynamics (renal artery trunk) in patients without signs and with signs of renal graft dysfunction in the long-term period following KT are presented in Table 1.

As can be clearly seen from the presented data, the parameters of renal artery resistance and marked velocity gradient in patients with relatively normal serum creatinine levels (group 1) in the long-term period following KT, despite the differences from the reference values, did not reach statistical significance, which was not the case for acceleration time dynamics and TAMX, as statistically significant differences were noted in the CKT recipients: 23.08 % (P < 0.05) and 13.5 % (P < 0.05), respectively.

According to the obtained results, in patients with efficient kidney transplant function after LRKT (group 1), the PSV in the renal artery was 32.89 % higher than in those with renal dysfunction (group 2) after LRKT. After CKT, the differences between groups of patients with and without renal dysfunction (group 1 and 2) were 2.08 %, not being clinically significant. However, the greatest differences were revealed in such indicators as EDV in the renal artery and TAMX. EDV in the renal artery was 76.77 % higher in patients with efficient renal function (group 1) after LRKT than in those with impaired kidney transplant function (group 2). The same indicator was 44.83 % higher in patients with efficient kidney transplant function (group 1) compared to those with kidney transplant dysfunction (group 2) after CKT. Regarding such an indicator

Table 1. The Doppler spectrum parameters of the renal transplant blood flow in patients with efficient and impaired depuration function (renal artery trunk) in the long-term period following KT

| Indicators, units | Efficient function of depuration (group 1) | Impaired function of depuration (group 2) |
|-------------------|-------------------------------------------|------------------------------------------|
|                   | Living related kidney transplants (n = 16), M ± m | Cadaveric kidney transplants (n = 10), M ± m | Living related kidney transplants (n = 16), M ± m | Cadaveric kidney transplants (n = 10), M ± m |
| Peak systolic velocity (PSV), renal artery trunk, cm/s | 94.75 ± 8.06 | 79.5 ± 5.04 | 71.30 ± 6.65* | 77.88 ± 5.43* |
| End-diastolic velocity (EDV), renal artery trunk, cm/s | 35.00 ± 2.94 | 31.95 ± 1.97 | 19.90 ± 1.58* | 22.06 ± 1.77* |
| Systolic/diastolic ratio (SDR), relative units | 2.76 ± 0.17 | 2.51 ± 0.10 | 3.69 ± 0.34* | 3.60 ± 0.12* |
| Pulsatility index (PI), renal artery trunk, relative units | 1.08 ± 0.07 | 1.0 ± 0.05 | 1.44 ± 0.12* | 1.44 ± 0.04* |
| Resistance index (RI), renal artery trunk, relative units | 0.62 ± 0.02 | 0.6 ± 0.02 | 0.71 ± 0.02* | 0.72 ± 0.01* |
| Acceleration time (AT), sec | 0.13 ± 0.01 | 0.16 ± 0.01 | 0.14 ± 0.01 | 0.12 ± 0.01* |
| TAMX, cm/sec | 54.87 ± 4.63 | 47.46 ± 2.76* | 35.48 ± 2.38* | 39.02 ± 2.77* |

* The statistical significance of differences in comparison to the group with efficient depuration function is less than 0.05.
Table 2. The Doppler spectrum parameters of the renal transplant blood flow in patients with efficient and impaired depuration function (interlobar arteries) in the long-term period following KT

| Indicators, units | Efficient function of depuration (group 1) | Impaired function of depuration (group 2) |
|-------------------|-------------------------------------------|------------------------------------------|
|                   | Living related kidney transplants (n = 16), M ± m | Cadaveric kidney transplants (n = 10), M ± m |

**Upper segment**
- Peak systolic velocity (PSV), interlobar branches of the renal arteries cm/s: 32.13 ± 2.23 vs. 30.1 ± 1.04, difference was 54.65 % favor of the group with normal renal function.
- End-diastolic velocity (EDV), interlobar branches of the renal arteries, cm/s: 13.38 ± 1.03 vs. 12.9 ± 0.64, difference was 53.79 % favor of the group with normal renal function.
- Systolic/diastolic ratio (SDR), relative units: 2.44 ± 0.08 vs. 2.43 ± 0.11, difference was 90.27 % favor of kidney transplant without kidney transplant dysfunction.
- Pulsatility index (PI), interlobar branches of the renal arteries, relative units: 0.94 ± 0.03 vs. 0.92 ± 0.04, difference was 1.29 % favor of the group with normal renal function.
- Resistance index (RI), interlobar branches of the renal arteries, relative units: 0.58 ± 0.01 vs. 0.58 ± 0.02, difference was 0.88 % favor of the group with normal renal function.
- Acceleration time (AT), s: 0.14 ± 0.01 vs. 0.13 ± 0.01, difference was 8.88 % favor of the group with normal renal function.
- TAMX, cm/s: 19.99 ± 1.38 vs. 19.05 ± 0.63, difference was 4.77 % favor of kidney transplant without kidney transplant dysfunction.

**Middle segment**
- Peak systolic velocity (PSV), interlobar branches of the renal arteries cm/s: 33.0 ± 2.8 vs. 30.9 ± 1.62, difference was 54.65 % favor of the group with normal renal function.
- End-diastolic velocity (EDV), interlobar branches of the renal arteries, cm/s: 12.36 ± 0.96 vs. 13.0 ± 0.82, difference was 67.75 % favor of kidney transplant without kidney transplant dysfunction.
- Systolic/diastolic ratio (SDR), relative units: 2.7 ± 0.1 vs. 2.41 ± 0.12, difference was 30.16 % favor of the group with normal renal function.
- Pulsatility index (PI), interlobar branches of the renal arteries, relative units: 1.02 ± 0.04 vs. 0.93 ± 0.04, difference was 2.00 % favor of the group with normal renal function.
- Resistance index (RI), interlobar branches of the renal arteries, relative units: 0.62 ± 0.01 vs. 0.58 ± 0.02, difference was 6.67 % favor of the group with normal renal function.
- Acceleration time (AT), s: 0.13 ± 0.01 vs. 0.14 ± 0.01, difference was 6.67 % favor of the group with normal renal function.
- TAMX, cm/s: 19.03 ± 1.47 vs. 19.4 ± 1.15, difference was 27.00 % favor of kidney transplant without kidney transplant dysfunction.

**Lower segment**
- Peak systolic velocity (PSV), interlobar branches of the renal arteries cm/s: 32.06 ± 1.73 vs. 31.3 ± 1.24, difference was 23.05 % favor of the group with normal renal function.
- End-diastolic velocity (EDV), interlobar branches of the renal arteries, cm/s: 13.31 ± 0.78 vs. 12.9 ± 0.46, difference was 19.80 % favor of the group with normal renal function.
- Systolic/diastolic ratio (SDR), relative units: 2.43 ± 0.09 vs. 2.42 ± 0.12, difference was 27.02 % favor of the group with normal renal function.
- Pulsatility index (PI), interlobar branches of the renal arteries, relative units: 0.94 ± 0.04 vs. 0.93 ± 0.05, difference was 1.07 % favor of the group with normal renal function.
- Resistance index (RI), interlobar branches of the renal arteries, relative units: 0.58 ± 0.01 vs. 0.58 ± 0.02, difference was 0.88 % favor of the group with normal renal function.
- Acceleration time (AT), s: 0.13 ± 0.01 vs. 0.18 ± 0.01, difference was 31.00 % favor of the group with normal renal function.
- TAMX, cm/s: 19.99 ± 0.95 vs. 19.38 ± 0.65, difference was 32.80 % favor of the group with normal renal function.

*: the statistical significance of differences in comparison to the group with efficient depuration function is less than 0.05.

Discussion

Summing up the data obtained, it can be concluded that TAMX is not only an important parameter and a key marker of dysfunction at the initial stage of renal failure development, but it also adequately reflects the transplanted kidney functional status in recipients without transplant dysfunction.

Clinical manifestations of rejection are less pronounced, and, as a rule, become evident only due to an increase in serum creatinine level or even occur subclinically. In recent years, studies have shown that rejection is the main cause of transplanted kidney failure in the long-term period following KT, and late rejection occurs with the humoral immunity activation in most cases.
Activation of the humoral immunity in the late rejection episodes development more determines its resistance to therapy and poor prognosis regardless of the morphological variant of the latter. Traditionally, acute and chronic humoral rejection are distinguished; however, in essence, these variants of rejection are stages of the same process, that begins with anti-donor antibodies production which form complexes with antigens on the surface of endothelium, that leads to the classical pathway of complement system activation resulting in inflammation development (acute rejection) and further vascular repair in the smallest blood vessels (chronic rejection) [9].

Despite the development of new, more powerful immunosuppressive agents, it has not yet been possible to completely overcome the problem of renal transplant rejection. At the same time, late rejection episodes, which despite the treatment are the most serious for prognosis, are manifested in further progression of kidney transplant dysfunction in most cases.

For a long time, the nature of such differences in the rejection course depending on the time of occurrence remained unclear, and only in recent years, it was possible to prove that humoral immunity activation plays an important role in the development of late rejection episodes. According to the data from the DeKAF study, more than 50 % of patients with late dysfunction showed signs of acute or chronic kidney transplant rejection, and 57 % of these patients showed signs of the humoral immunity activation [10].

Today, clinical diagnosis of graft rejection is based on histological examination of kidney transplant biopsies. Since a biopsy is usually performed only in increased creatinine levels, the beginning of rejection treatment is delayed for several days or even weeks. Such a delay contributes to tissue damage reducing graft survival. In addition, a biopsy is an invasive procedure, which cannot be performed as often as necessary for careful monitoring, so it is not an ideal method for the diagnosis of rejection.

It is important to note that the Doppler evaluation of transplant vessels to detect complications of kidney transplantation and the specificity of kidney transplant pathology has turned out to be a promising method of examination. Color mapping and pulsed dopplerography are considered as the most accurate in demonstrating a kidney transplant state and are the gold standard for monitoring the kidney transplant state and hemodynamic changes both in the early and long-term postoperative periods.

Conclusions

1. We conducted a comparative analysis of complex ultrasound indicators (grayscale with color and spectral Doppler sonography) of blood flow that did not reveal significant differences between the patients with efficient depuration function after LRKT and CKT in the late post-operative period.

2. In patients after LRKT and CKT with impaired depurative function, lower TAMX values were recorded in comparison to patients without renal dysfunction, which can be a reliable diagnostic criterion and an important prognostic predictor for renal transplant dysfunction development.

3. The obtained data reliably indicate that if TAMX of interlobar arteries less than 15 cm/s, the probability to reveal the clinical signs of renal allograft dysfunction is more than 90 %, regardless of the renal segment evaluation in recipients in the long-term period after KT.

Prospects for further research. This study would provide a perspective basis for proposed Doppler sonographic parameters using as possible criteria for impaired graft perfusion. These data will allow for a comparative analysis of Doppler indices in recipients with graft dysfunction, to define ultrasound criteria for kidney transplant dysfunction, and thereby to improve diagnosis and prolong graft survival.

Conflicts of interest: author has no conflict of interest to declare.

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