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NGAL As A Biomarker For Early Detection Of Renal Allograft Dysfunction In Living Donor Transplantation

Norkuziev, Sherali Safaralievich.[1], Kurbanov Obid Makhsudovich.[2], Muminov Javokhir Asliddinovich. [1], Ismatov Tuichiboy Akhrorkulovich [1], Norkabilov Alisher Norbekovich [1], Olimov Ismoilkhon Zhamolkhon ugli[1] Mamatmurodov Anvar Abdusaidovich [1]

[1] Samarkand regional multidisciplinary medical center

[2] Bukhara State Medical Institute

Resume,

This open-label, randomized, retrospective, comparative study investigated the prognostic value of urinary NGAL (neutrophil gelatinase-associated lipocalin) levels in predicting renal allograft dysfunction in 18 patients who underwent living related donor kidney transplantation at the Samarkand Regional Multidisciplinary Medical Center between January and September 2024. Participants were followed up for three months after transplantation. Renal graft dysfunction (DGF) was defined based on the criteria of Halloran et al. Dynamic measurements of NGAL, serum creatinine, and urea during the first week of the postoperative period showed a significant decrease in these parameters in 17 of 18 patients. Graft dysfunction was observed in one case, which was accompanied by abnormalities. The data obtained confirm that NGAL is a useful biomarker for the early diagnosis and monitoring of renal graft function. This method may improve the prediction of complications and optimize the clinical management of patients after kidney transplantation.

Key words: NGAL, kidney transplantation, graft dysfunction

Relevance

Acute kidney graft dysfunction can be caused by ischemic injury or immunologic injury, resulting in serious consequences in both the short and long term [1,4,10,15,21] . We desperately need biomarkers of immune and non-immune injury at different time points during the transplantation process, starting from potential kidney donors where acute kidney injury may remain undetected, in the early post-transplant periods to predict acute graft dysfunction due to various causes, and during long-term follow-up to predict chronic histologic changes [2,5,11,18,19]. The implementation of these new biomarkers may improve the sensitivity of diagnosis and monitoring of kidney injury in kidney transplant recipients. Traditionally, acute graft dysfunction is diagnosed by measuring serum creatinine concentration [3,6,7,13,16,20]. Unfortunately, an increase in serum creatinine is a late sign of kidney injury [3,8,14,12,17,2 2]. It indicates, rather than predicts, injury. Treatment to be effective must be initiated very early after the initiating insult, long before serum creatinine begins to rise. Fortunately, new technologies such as functional genomics and proteomics have revealed new candidates that are emerging as potentially useful biomarkers of acute kidney allograft injury. The most promising of these is neutrophil gelatinase-associated lipocalin (NGAL). The prognostic value of NGAL in kidney transplantation has been widely reported in the literature [5,9,11,17,23]. However, the authors of the scientific studies on the prognostic value of NGAL have mainly been studied in patients after cadaveric kidney transplantation, and its prognostic value in living donor kidney transplantation remains out of sight. Recently, we found that recipient urine NGAL (U-NGAL) measured on the first morning after transplantation predicted dysfunctional graft function (DGF) of living donor kidneys, especially in cases where early graft function (EGF) was expected based on urine output and decrease in plasma creatinine concentration. In addition, recipient U-NGAL could predict DGF lasting more than two weeks [3,6,14,16,24].

The aim of the study is to evaluate the prognostic significance of urinary NGAL (neutrophil-granulocyte leukocyte antigen) levels in early diagnosis and prediction of renal allograft dysfunction in living donor recipients.

Materials and methods.

An open-label, randomized, retrospective, comparative study in 18 patients who underwent living related donor renal allotransplantation performed at Samarkand Regional Multidisciplinary Medical Center between January and September 2024. Included patients were their first living donor kidney transplant

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April 2025

recipients and were followed for 3 months postoperatively. The primary recipient outcome variable was the onset of graft function after transplantation. DGF was defined as described by Halloran *et al.*: oliguria <1 L/24 h for >2 days or plasma creatinine >500 μmol/L during the first week after transplantation or the need for more than one dialysis session during the first week after transplantation.

Postoperative urine samples were collected from patients in the morning after fasting. Samples on the first postoperative day were collected approximately 8–12 hours after kidney transplantation. Samples were collected daily from the first to the seventh day after kidney transplantation. U-NGAL assays were performed using a standardized clinical platform (ARCHITECT analyzer; Abbott Diagnostics, Abbott Park, IL, USA) as described previously. On the eve of surgery, all donors were tested for serum nitrogenous waste and urine NGAL to accurately assess the functional status of the kidney graft. The quantitative NGAL indicator in donor urine ranged from 8 to 18 ng/mL. In our study, we established the upper limit of NGAL in urine in healthy patients to 20 nm/mL.

The main clinical characteristics of the kidney transplant patients are shown in the table below. Eighteen patients were included in the study, including 11 men and 7 women, and the mean age was 29 years (range 19–52). Among the 18 patients, the most common complications were renal hypertension (57%) and renal anemia (83%). All patients had end-stage renal disease before transplantation. In all patients, chronic renal failure developed against the background of chronic glomerulonephritis.

Table 1. Clinical data of 18 kidney recipients

Patient characteristics	Data by numbers n = 18, n %
Age (years), median (range)	**29 (19-52)**
Floor	
Male	11
Female	7
Main diseases	
Chronic glomerulonephritis	18
Associated diseases	
Renal hypertension (%)	11
Renal anemia (%)	16
Substitution therapy regimen	
Connected to hemodialysis	14
Not connected to substitution therapy	3

Trends in kidney function-related parameters during one week after kidney transplantation. Our study included 18 patient, primary kidney transplantation. The data showed that within one week after kidney transplantation, urinary NGAL, serum creatinine and urea showed a significant decrease in 17 recipients. In 1 recipient, renal graft dysfunction was observed in the postoperative period. In this case, the urinary NGAL, serum creatinine and urea dynamics were different from the other recipients.

Renal function test values in recipients prior to surgery
(Pooled average of NGAL in 18 recipients)
Urea (μmol/l) 19.5 (14.50-29.5)
Serum creatinine (μmol/l) 970.5 (655.4-1950.5)
Urinary NGAL (ng/ml) 1130(870-1805)

Table 2. Indicators of renal transplant function tests in 17 patients with no signs of dysfunction during the postoperative period.

Indicators / day	**1d**	**2d**	3 **d**	4 **d**	5 **d**	6 **d**	7 **d**
Urinary NGAL (ng/ml)	1041,5 (382.59, 1580,43)	243.74 (223.63, 391.74)	141.98 (56.49, 182.82)	32.23 (18.72, 41,59)	25.82 (16.4 32.89)	21,26 (16.9, 23.5)	21.4 (14.96, 21.63)
Creatinine (μmol/l)	260.5, (160.5,	180.5 (140.5,	144.4 (110.6,	124.6, (94.4,	114.12 (86.3	112.9 (87.25,	90.76 (63.25,

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April 2025

	460.5)	256)	173.5)	136.6)	140.0)	129.75)	120.25)
Urea (µmol/L)	14.5 (12.50, 16.4)	13.7 (11.4, 14.7)	12.4 (10.5, 14.0)	10.5 (8.7, 11.4)	8.7 (8,1, 9.7)	7.9 (7.4, 8,8)	14.5 (12.50, 16.4)

The postoperative period in 17 patients proceeded without complications, and the patients were discharged on the 9-10th postoperative day. In one patient, dysfunction of the transplant was observed in the postoperative period.

Clinical Observation.

Patient H.S., 47 years old, diagnosed with chronic glomerulonephritis, end-stage renal failure, had been undergoing program hemodialysis treatment for the past 6 months. In our center, after conducting the appropriate examinations, a decision was made to perform surgical treatment - kidney transplantation from a living related donor.

The patient's 52-year-old sister was selected as the donor. HLA typing of the donor and recipient revealed three mismatched antigens. The serologic cross-match was 13%. The panel of reactive antibodies for HLA class I and HLA class II was negative. The test for the presence of donor-specific antibodies (DSA) performed using the Luminex system was also negative. The general condition of the donor and her renal function indices corresponded to the established standard criteria (SCF 96 mmol/ml).

Table 3. Data of preoperative immunological examination of donor and recipient

Parameters		Donor	Recipient
Blood group		0 (1)	0 (1)
HLA typing		A*01, A*01, B*07, B*50 DRB 1*15 DRB 1*07	A*01, A*03, B*07, B*27 DRB 1*15 DRB 1*16
Incompatible HLA antigens		B*50, B*27, DRB 1*0 7 DRB 1*16 B * 50	
Level of pre-existing antibodies	Anti HLA class 1 Anti HLA class 11		NEGATIV NEGATIV
Cross match			13%
DSA			NEGATIV

In accordance with the protocol for preparing the recipient for surgery, three plasmapheresis sessions were performed in the preoperative period. Due to this, the risk of immunological complications seemed insignificant, and it was decided to use monoclonal antibodies - Semulect in a standard dosage as induction immunosuppression.

Methylprednisolone 750 mg was administered intravenously before transplant reperfusion. On the eve of surgery, the recipient's serum creatinine level was 590 µmol/L, urea level was 20.4 mmol/L, and urine NGAL level was 1670 ng/mL.

The kidney transplantation was performed using the standard technique: the left kidney was transplanted into the right iliac region. No intraoperative complications were noted. After reperfusion, the transplant acquired a satisfactory color and turgor, and urine flow was observed at a good rate. The cold ischemia period was 68 minutes.

ISSN NO: 2770-2936
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From the first day, triple immunosuppressive therapy was prescribed: tacrolimus - 8 mg per day (target blood concentration level of 0.12 mg/kg), methylprednisolone - 16 mg per day intravenously, mycophenolate - 2000 mg per day. Diuresis for the first 12 hours was 6400 ml. The serum creatinine level was 605 μmol/l, urea - 21.5 mmol/l, NGAL - 1870 ng/ml. In the next 12 hours, a sharp decrease in the rate of urine output to 1450 ml was observed, and during the first postoperative week, daily diuresis did not exceed 450 ml. Hemodialysis sessions were started on the fourth day after the operation.

Daily ultrasound examinations of the transplant showed the patency of the vascular anastomoses, the resistance index on the segmental and arcuate arteries was within 0.8–0.9. The volume of the transplant tended to increase.

During the first postoperative week, 4 injections of thymoglobulin at a dose of 50 mg (on days 0, 1, 2, 3), a single injection of rituximab (500 mg intravenously), and an injection of 10% serum immunoglobulin (100 ml intravenously) were performed. Laboratory tests during the first week remained within the following limits: serum creatinine level - 490-560 μmol/l, urea - 16-21 mmol/l, urine NGAL - 1600-1950 ng/ml, blood tacrolimus concentration - 6.5-8.0 ng/ml. On the seventh day, a puncture biopsy of the graft was performed. Histological examination showed a picture of hyperacute rejection. Immunohistochemistry revealed widespread linear fixation of C3d and C4d components in the walls of most peritubular capillaries. Screening for anti-HLA antibodies by Luminex on the day of biopsy revealed antibodies to HLA class I and II. The mean fluorescence intensity of microspheres with incompatible HLA antigens ranged from 3000 to 12,000 units.

On the same day, a three-day pulse therapy with methylprednisolone at a dose of 500 mg per day intravenously was started. The following morning, a plasmapheresis session with a replacement volume of 2700 ml was performed, after which 5 g (100 mg/kg) of serum immunoglobulin (Gamunex) was administered intravenously. Over the next week, two more plasmapheresis sessions and three administrations of immunoglobulin at a dose of 5 g were performed. Beginning on the 14th day, an increase in urine volume to 2.5 L was observed, and laboratory parameters began to normalize. On the 14th day, laboratory data were as follows: serum creatinine - 462 μmol/l, urea - 16.4 mmol/l, urinary NGAL - 32.4 ng/ml. On the 20th day, the serum creatinine level was 160 μmol/L, urea was 8.3 mmol/L, and urinary NGAL was 18 ng/ml.

The patient was discharged home on the 22nd day after the surgery. In order to prevent re-formation of antibodies, rituximab infusion at a dose of 350 mg was performed on the 42nd day. At the time of drug administration, the patient's condition was satisfactory: serum creatinine level was 82 μmol/l, urea - 7.6 mmol/l, urinary NGAL - 12 ng/ml.

Conclusions:

- ✓ The study demonstrated the high prognostic value of urinary NGAL (neutrophil-granulocyte leukocyte antigen) for the early diagnosis of renal transplant dysfunction in recipients of kidneys from living related donors.
- ✓ A dynamic decrease in urinary NGAL levels during the first week after transplantation correlated with improved renal graft function in most patients, highlighting the importance of monitoring this biomarker.
- ✓ In one recipient with renal graft dysfunction, abnormal urinary NGAL, serum creatinine, and urea were observed, supporting the role of NGAL as an indicator of complications.
- ✓ The obtained data indicate that the use of NGAL can significantly improve the management of patients after kidney transplantation, facilitating timely diagnosis and prevention of complications.

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ISSN NO: 2770-2936
April 2025

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