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LABORATORY STUDY

Plasma Glutathione Peroxidase Activity in Kidney Recipients with and without Adverse Outcome

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Abstract

Kidney function is routinely monitored utilizing classic biochemical parameters including serum or plasma creatinine (Cr), and blood urea nitrogen (BUN) concentrations. This study demonstrates that the simultaneous assessment of plasma glutathione peroxidase (pGPx) and Cr levels provides a better strategy for the immediate follow-up of kidney function in organ recipients. Kidney recipients (Krs; $n = 22$) were recruited. Blood sampling schedule commenced at day 1 (pre-transplantation) and post-transplantation days (i.e., everyday from 1 until day 14, and thereafter on days 21, 28, 35, 42, 49, and 56). pGPx was measured spectrophotometrically. Candidates for transplantation exhibited lower pGPx than control subjects (42 ± 24 vs. 143 ± 31 U/L; $p < 0.005$). In Krs with a stable post-transplant outcome, pGPx increased to a maximum at day 28 (214 ± 61 U/L). In a Kr diagnosed with acute tubulonecrosis, pGPx provided a better predictive value (threefold increase) than Cr. In a Kr diagnosed with acute rejection, the increment in Cr values was found to be more pronounced than in pGPx values. The pGPx test is simple, inexpensive and automatable, and should be a valuable diagnostic tool of kidney function in organ recipients with and without troublesome outcome for the follow-up during hospitalization period.

Keywords: Glutathione peroxidase, kidney transplantation, end-stage renal disease, antioxidant enzymes

INTRODUCTION

Kidney transplantation is a selected treatment for patients with end-stage renal disease (ESRD).¹ It has been recognized that renal recipients from living donors have longer survival rates than in those from cadaveric donors.^{2,3} However, the underlying mechanism is not well established.^{4–7} Kidney function is routinely monitored utilizing classic biochemical limits including serum or plasma creatinine (Cr), and blood urea nitrogen (BUN) concentrations.

Glutathione peroxidase (GPx) is a family of selenoproteins.^{8,9} GPx exists in various isoenzymes including cytosolic (cGPx), gastrointestinal (GI-GPx), plasma (pGPx) and phospholipid hydroperoxide (PHGPx).¹⁰ With the exception of PHGPx, which is a monomer, all other GPx isoenzymes consist of four identical subunits. Each subunit contains a single seleno-cysteine residue that is essential for the catalytic activity of GPx.^{11,12} GPx catalyzes the reduction of organic hydroperoxides (ROOH)

and hydrogen peroxide (H_2O_2) utilizing glutathione as a reducing agent. Thus, one of the physiological functions of GPx is the protection of extracellular fluid components and cell surfaces against peroxide-mediated damage.^{11–13}

In the kidney, pGPx is highly abundant in the S1 and S2 segments of the proximal tubules.^{14,15} Lower pGPx activity (39–52%) has been reported in nondialyzed individuals with renal failure^{16–19} and those either on hemodialysis^{18,20,21} or on Continuous Ambulatory Peritoneal Dialysis (CAPD)^{21,22} when compared with control subjects. The decline in pGPx activity in subjects with renal failure has been linked to the impairment of pGPx biosynthesis in the kidney.¹⁹ Whitin et al.²³ analyzed pGPx activities in kidney recipients (Krs) subdivided according to organ source [i.e., living related donors (LRD) or cadaveric donors (CD)]. It was found that pGPx activities in Krs were improved rapidly within 1 week after organ replacement therapy. Two to three weeks post-transplantation, Krs exhibited higher pGPx

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activity than healthy control subjects. The trends for the improvement in pGPx activities post-surgery were similar when comparing organs from LRD or CD. Similarly, Zachara et al.²⁴ reported that pGPx activity was markedly improved immediately after transplantation. However, no difference was seen in the magnitude of pGPx activities between Krs and healthy controls.²⁵ These data indicate that monitoring pGPx activity in Krs may provide useful tool in detecting chronic allograft nephropathy (CAN), which is a leading cause of organ failure during the first year after renal transplantation.^{26,27}

The aims of this cross-sectional study were to explore the relationships between pGPx activities and the concentrations of serum Cr in organ recipients with and without adverse outcome. This study extends beyond previous investigations in the term that a homogenous group of Krs has been enrolled and that tighter measurement has been conducted during hospitalization period.

MATERIALS AND METHODS

Reagents

Reduced glutathione (GSH), glutathione reductase (GR), and nicotine amid adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Corporation (Dorset, UK). Tris-HCl buffer, NaN₃, ethylenediaminetetraacetic acid (EDTA) and H₂O₂ were from E. Merck (Darmstadt, Germany). Kits for routine biochemical parameters were obtained from Pars Azmon Company (Tehran, Iran).

Studied Population

The recipients of first kidney (age range: 30–60 years, $n = 22$) were recruited through the Department of Nephrology, Imam Khomeini Teaching Hospital, Urmia University of Medical Sciences, Urmia, Iran. This study was commenced at April 2005 and terminated at September 2007. Inclusion criteria were males of age 30–60 years and recipients of first kidney. Exclusion criteria were diabetes, autoimmune disease, acute phase and/or chronic inflammation, and antioxidant oxidant supplementation 2 months pre-admission. The participants received a cocktail comprising Cyclosporine (5 mg/kg/day), Azathioprine (1 mg/kg/day), and Prednisolone (1 mg/kg/day) as immunosuppressive therapy (IT). For comparison, volunteer subjects (age and sex matched; $n = 25$) with no history of hypertension, diabetes, or renal disease were employed as the control group. Candidates for kidney transplantation gave their written consent after explaining the study objectives. This study was approved by the ethical committee at Urmia University of Medical Sciences.

Blood Collection and Plasma Preparation

For routine biochemical measurement, morning blood sample (2 mL) was collected before IT intake. The sample was allowed to coagulate at room temperature for

30 min and serum was obtained by centrifugation at $2000 \times g$ for 10 min.

At the time of venipuncture for routine biochemical analyses, blood samples (2 mL) were also collected for measurement of pGPx activity. The sample was transferred into glass tube containing EDTA as an anticoagulant at a final concentration of 1 mg/mL. Sampling schedule commenced at day 1 (pre-transplantation) and post-transplantation days (i.e., everyday from 1 until day 14 and thereafter on days 21, 28, 35, 42, 49, and 56). Plasma was obtained by centrifugation at $2000 \times g$ for 10 min at 4°C. Aliquots (200 μ L) were transferred into Eppendorf tubes and subsequently stored at -80°C until analysis.

In the case of control group, blood samples were drawn after an overnight fast. The remaining procedures for blood separation were as described above.

Routine Biochemical Measurements

Glucose was measured using the hexokinase method. Uric acid was determined using a uricase-based assay. Creatinine was assessed employing a creatininase-based test. Total cholesterol was measured using the Cholesterol-C high-performance CHOD-PAP assay. Triacylglycerols was determined using the enzymatic method. Inorganic phosphor and calcium were measured spectrophotometrically. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were monitored by coupling transaminase reactions. A continuous-monitoring technique based on the molar absorptivity of *p*-nitrophenol was used for the determination of alkaline phosphatase (ALP) activity. Sodium and potassium were determined flame photometrically. Urea was measured using colorimetric method by employing diacetyl monoxime.

Measurement of GPx Activity

pGPx activity was measured using coupled enzymatic reaction at 25°C as was previously described by Paglia and Valentine.²⁸ Briefly, coupling reaction was prepared in Tris-HCl buffer (50 mM; pH 7.6) comprising GSH, NADPH, EDTA, NaN₃ and GR, at a final concentration of 1 mM, 0.2 mM, 2 mM, 1 mM and 100 units, respectively. The reaction was initiated by adding H₂O₂ at a final concentration (1 mM) and monitored for 3 min at 340 nm. One unit of the enzyme activity was expressed as 1 μ mol of NADPH oxidized per minute, and the result was expressed as unit per liter of plasma.

Statistical Analysis

Analyses were performed using the SPSS software package for windows (version 13; SPSS, Chicago, IL, USA). Continuous data were expressed by the arithmetical mean \pm SD. Paired *t*-test was used for comparison of the data from different sampling points. Differences between groups were analyzed using the *t*-test for two independent samples. Pearson's correlation analysis was

used to assess associations between parameters. A p -value <0.05 was considered statistically significant.

RESULTS

Clinical and biochemical characteristics of Krs (i.e., at pre-operation day and at days 1, 3, 7, and 14 post-operation) are presented in Table 1. As can be seen in Table 1, body weight and systolic blood pressure were slightly lower on day 14 post-operation when compared with pre-operation day. Diastolic blood pressure was also found to be lower but the difference did not reach statistical significance. The Krs exhibited lower levels of Cr, BUN, potassium, and phosphorus ($p < 0.05$) when compared with baseline. Similarly, a decline in ALP activity was seen post-transplantation but the differences failed to reach statistical significance. On the contrary, a slight enhancement in cholesterol and TG contents was noticed after surgery when compared with baseline.

A marginal increase in GPT activity was also detected after surgery.

Figure 1 shows the alteration in pGPx activity in Krs with stable post-operation outcome. The mean value for pGPx activity among the candidates for kidney transplantation was markedly lower than among the control subjects (41.9 ± 24.2 vs. 143.35 ± 30.8 U/L; $p < 0.005$). No differences were observed in pGPx activity between the first day of pre-operation and the first day post-operation. However, significant elevations in pGPx activity were noticed from the second day onwards. pGPx activity reached a maximum on day 28 post-operation (214.2 ± 61.0 U/L). A 12% drop in pGPx activity was noted on day 35 when compared with day 28 (188.6 ± 63.2 vs. 214.2 ± 61.0 U/L; $p > 0.05$). From day 35 onwards, pGPx activity remained fairly unchanged.

Figure 2 shows the concomitant changes in pGPx activity and Cr levels in Krs with stable post-operative

Table 1. The profile for pGPx activity in Krs with stable post-operative outcome ($n = 20$).

| Parameters | Day | | | | | p |
|-------------------|---------------------|---------------|---------------|---------------|----------------|--------|
| | Pre-transplantation | 1 | 3 | 7 | 14 | |
| Weight (kg) | 66.3 ± 13.2 | 64.4 ± 13.7 | 65.8 ± 13.3 | 63.6 ± 13.1 | 63.6 ± 13.7 | <0.05 |
| BPs (mmHg) | 147.4 ± 23 | 159.6 ± 22.1 | 159.9 ± 16.3 | 144 ± 19.2 | 138.6 ± 15.6 | <0.05 |
| BPd (mmHg) | 90.2 ± 20.22 | 90.52 ± 11.11 | 87.5 ± 11.11 | 87.7 ± 10.2 | 84.26 ± 11.2 | NS |
| FBS (mg/dL) | 78.148 ± 21.2 | 267.92 ± 90 | 127.92 ± 43.2 | 87.03 ± 25.5 | 89.81 ± 34.3 | NS |
| Chol (mg/dL) | 153.33 ± 58.3 | NA | 126.15 ± 32.3 | 157.38 ± 18.6 | 190.41 ± 35.3 | NS |
| TG (mg/dL) | 155.33 ± 69.6 | NA | 75.15 ± 44.4 | 145.46 ± 71.2 | 180.83 ± 104.3 | NS |
| Cr (mg/dL) | 10.83 ± 5.2 | 4.89 ± 2.1 | 1.95 ± 0.7 | 1.59 ± 0.6 | 1.72 ± 0.5 | <0.005 |
| BUN (mg/dL) | 53.81 ± 18.8 | 32.96 ± 12.4 | 27.07 ± 10.4 | 36 ± 17.3 | 35.96 ± 20.1 | <0.005 |
| Uric acid (mg/dL) | 5.25 ± 0.8 | NA | 3.77 ± 0.18 | 5.34 ± 2.1 | 5.67 ± 3.3 | NS |
| Na (meq/L) | 136.04 ± 5.1 | 130.40 ± 5.1 | 134.55 ± 5.2 | 136.70 ± 5.3 | 135.8 ± 5.1 | NS |
| K (meq/L) | 7.8 ± 1.1 | 4.27 ± 0.84 | 4.02 ± 0.56 | 3.98 ± 0.6 | 4.15 ± 0.71 | <0.05 |
| P (mg/dL) | 4.4 ± 1.49 | NA | 3.5 ± 1.3 | 2.3 ± 1.4 | 2.77 ± 0.14 | <0.05 |
| Ca (mg/dL) | 8.08 ± 2.7 | NA | 8.21 ± 0.75 | 8.93 ± 1.16 | 8.56 ± 0.66 | NS |
| GOT (U/mL) | 27.22 ± 11.1 | NA | 31.77 ± 10.2 | 30.23 ± 9.8 | 30.92 ± 16.6 | NS |
| GPT (U/mL) | 28.55 ± 9.2 | NA | 25.61 ± 10.2 | 47.92 ± 25.2 | 60.27 ± 52.1 | NS |
| ALP (U/mL) | 176.88 ± 125.2 | NA | 119.46 ± 92.3 | 120.77 ± 68.6 | 129.92 ± 62.2 | NS |

Notes: p -Value (day 1 pre-transplantation vs. day 14 post-transplantation). Data are presented as mean ± SD. NS, not significant; BPs, blood pressure (systolic); BPd, blood pressure (diastolic); FBS, fetal bovine serum; Chol, cholesterol; TG, triacylglycerol; Cr, creatinine; BUN, blood urea nitrogen; Na, sodium; K, potassium; P, phosphorus; Ca, calcium; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ALP, alkaline phosphatase.

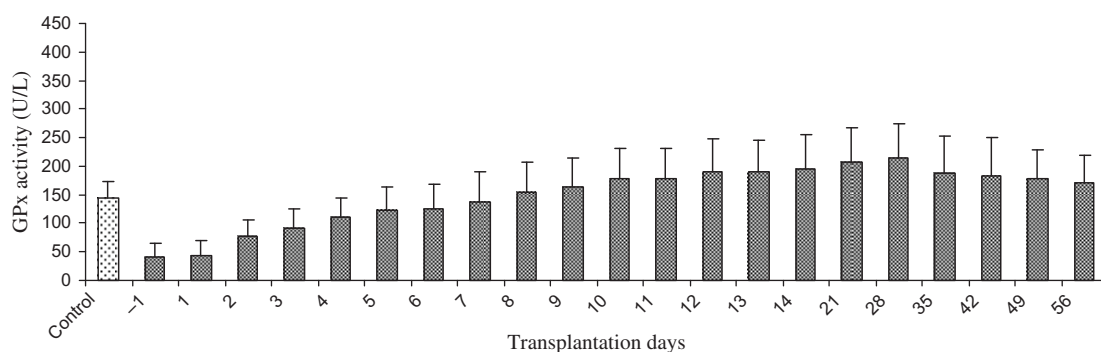


Figure 1. The profile for pGPx activity in kidney recipient with stable post-operative outcome ($n = 20$). Sample collection was commenced at day 1 pre-transplantation and terminated at day 58 post-transplantation. Data are presented as mean ± SD.

outcome. Negative correlations were seen between GPx activity and Cr levels ($r_p = -0.753$; $p < 0.005$).

The pattern for pGPx activity for a kidney recipient diagnosed with acute tubulonecrosis (ATN) is shown in Figure 3. The increments in pGPx up to day 9 post-surgery were similar to those with stable post-operative outcome. Thereafter, a marked enhancement in pGPx activity was observed on those with stable post-operative outcome. Measurement of pGPx activity was terminated on day 28 after surgery because the patient had to be

moved to another ward. The relationship between pGPx activity and the levels of Cr is demonstrated in Figure 4.

The trend for pGPx activity in a kidney recipient experiencing an episode of acute rejection (AR) is displayed in Figure 5. pGPx activity approached the expected values for those with stable post-operative outcome (i.e., 164 U/L) at day 7 post-operation. pGPx activity subsequently dropped to the expected values for control group ($143 \text{ U/L} \pm 30 \text{ U/L}$) and remained unchanged during the course of the investigation

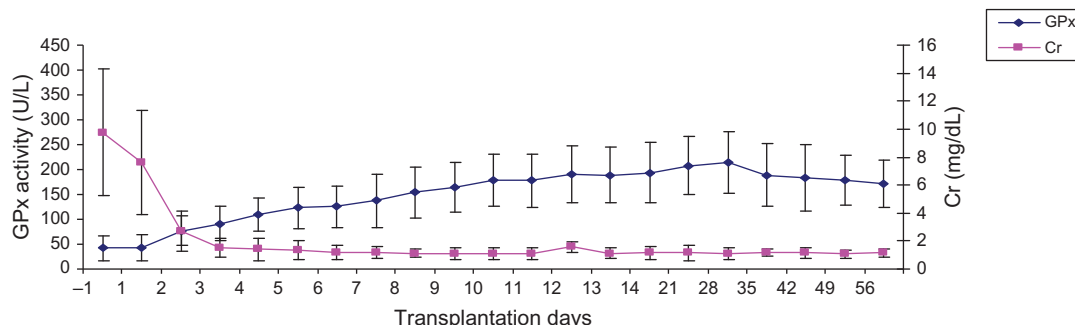


Figure 2. The relationship between pGPx activity and Cr levels in Krs with stable post-operative outcome ($n = 20$). Sample collection was commenced at day 1 pre-transplantation and terminated at day 58 post-transplantation. Data are presented as mean \pm SD.

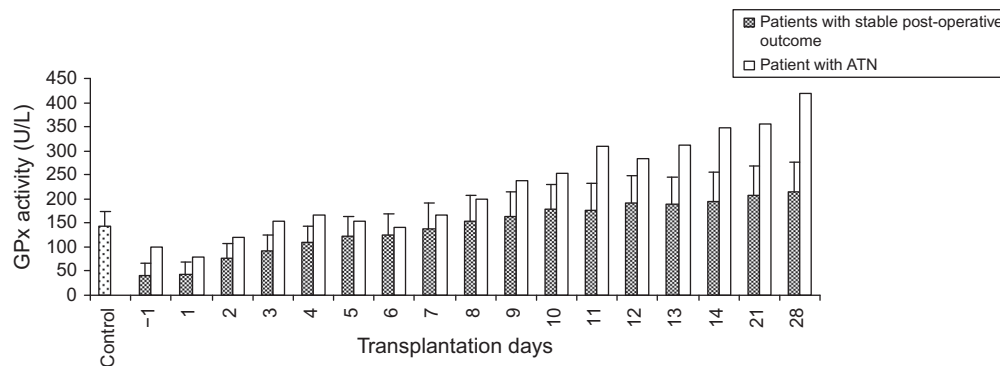


Figure 3. The profile for pGPx activity in kidney recipient with troublesome post-operative outcome (i.e., acute tubulonecrosis). Sample collection was commenced at day 1 pre-transplantation and terminated at day 28 post-transplantation.

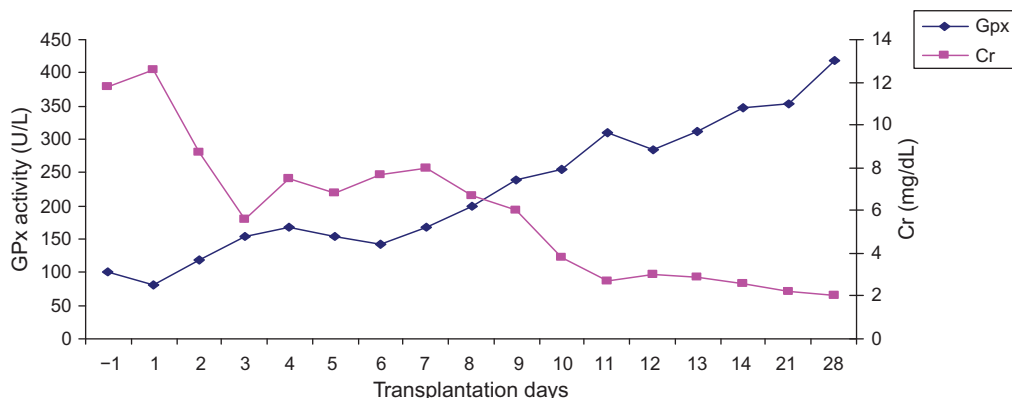


Figure 4. The relationship between pGPx activity and Cr levels in kidney recipient with troublesome post-operative outcome (i.e., acute tubulonecrosis). Sample collection was commenced at day 1 pre-transplantation and terminated at day 28 post-transplantation.

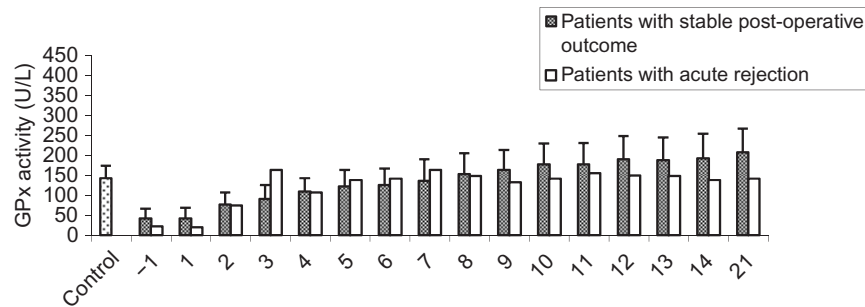


Figure 5. The profile for pGPx activity in kidney recipient with troublesome post-operative outcome (i.e., acute rejection). Sample collection was commenced at day 1 pre-transplantation and terminated at day 21 post-transplantation.

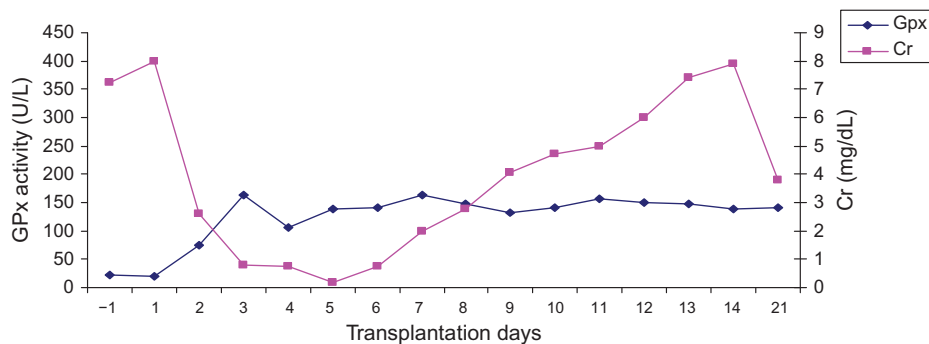


Figure 6. The relationship between pGPx activity and Cr levels in kidney recipient with troublesome post-operative outcome (i.e., acute rejection). Sample collection was commenced at day 1 pre-transplantation and terminated at day 21 post-transplantation.

(i.e., 21 days). The association between pGPx activity and Cr concentrations is shown in Figure 6.

DISCUSSION

Kidney replacement is the ultimate therapy for ESRD patients. Therefore, the diagnostic challenge is to detect early onset of renal dysfunction. Early diagnoses of renal dysfunction also imply better patient's outcomes assuming the effective therapeutic regimen are utilized. Although biopsy is invasive, it remains as the golden standard method for the evaluation of renal dysfunction. In this investigation, pGPx activity has been monitored as an index of kidney function in conjugation with Cr and BUN in renal recipients. From a practical point of view, the pGPx assay is simple, inexpensive, and automatable.

We report that the mean pGPx activity in the candidate for renal transplantation was 29% that in the control group. This value is in good agreement with those (31%) obtained by Zachara et al.²⁴ Respective value reported by Whitin et al.²³ was 50% employing *t*-butyl hydroperoxide as substrate in the pGPx assay instead of hydrogen peroxide.

This study has revealed that the largest increment in pGPx activity in renal recipients from living donors with stable post-operative outcome occurred between day 1 and day 2 post-transplantation. The mean pGPx activity

approached that of healthy control subjects within 9 days after transplantation. Maximum activity was recorded at day 28 post-surgery. These findings are in agreement with those previously reported by other investigators.^{23,24}

Additional support for the notion that the measurement of pGPx activity is a useful tool in detecting renal dysfunction is derived from examining the profile of pGPx activity in a kidney recipient experiencing an episode of ATN. As illustrated in Figure 5, kidney recipient with ATN exhibited higher pGPx activities than those with stable post-operative outcome. On day 28 post-surgery, the value for pGPx in patients was about three-fold higher than that in healthy control group. This may imply a greater demand on the kidney for increasing pGPx biosynthesis to maintain a normal renal function. On the contrary, the values for Cr on day 28 post-surgery revealed a marginal increase when compared with normal range and thus suggesting the importance of the simultaneous determination of pGPx and Cr as biomarkers of renal function in organ recipients.

The profiles for pGPx activity and Cr levels in a kidney recipient experiencing an episode of AR provide another proof for the usefulness of the inclusion of pGPx activity assay as complement for Cr during evaluation of kidney function post-surgery (Figures 5 and 6). The assessment of pGPx activity assay revealed that the trend of profile was similar to that of renal recipients with stable post-operative outcome albeit the magnitude of the activity

was marginally attenuated. On the other hand, the assessment of Cr seemed to provide better diagnostic values of renal function.

Simic-ogrizovic et al.²⁹ explored the usefulness of assay pGPx activity in long-survival graft without and with dysfunction 5 years after transplantation. They reported that the mean of pGPx activity in Krs with stable post-operative outcome was similar to that of the healthy control group. On the contrary, a subgroup with chronic rejection exhibited lower pGPx activity (25%) compared with the value for healthy control subjects. Taken together, these findings imply that the simultaneous assessment of Cr and pGPx activity should serve as a valuable tool for the follow-up of renal function after renal transplantation and/or during surveys of long-survival graft.

In this cross-sectional investigation, we have shown that the inclusion of pGPx assay as a clinical biomarker should improve detection of renal malfunction in organ recipients during hospitalization period as well as in surveys of long-survival graft. Further studies are needed to establish the power of the pGPx assay in distinguishing between individuals with adverse outcome from those with stable outcome.

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