Original Article

Effect of Acid-Base Balance on Cytokines Serum Levels and Short-Term Outcomes in Kidney Transplant Recipients; a Randomized Clinical Trial

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Abstract

Background: Control of blood acids and bases can help prevent many potentially life-threatening disorders in end stage renal disease (ESRD) patients. Aim of this study was to assess the effect of acid-base balance on cytokines serum levels and short-term outcomes in kidney transplant recipients.

Materials and Methods: In this randomized clinical trial study, 40 patients with end-stage renal disease aged 18 to 70 who had undergone a kidney transplant from a living donor in Modarres hospital during 2016-2017 were included. The primary outcomes measured in this study were sera levels of cytokine such as IL-2, IL-10, IFN-γ and BUN and Cr serum after the treatment of acidosis in kidney transplant recipients.

Results: Mean±SD of the patient's age was 42±12.6 years. Results showed that there is a significant difference in means of IL-2, IL-10, and IFN-γ between the intervention and control groups over the time (for all $p<0.05$). We also found that correction of acidosis occurred with reduces of IFN-γ to -1.74 in the intervention group compared to the group receiving saline ($P=0.011$); and reduction for IL-2 was -1.37 ($p=0.025$). The concentration of anti-inflammatory cytokine of IL-10 was increased to 2.85 ($P<0.001$).

Conclusion: The results clearly suggest that correction of acidosis in renal transplant patients during surgery helps improve the performance of allograft in the short run; however, more studies are recommended, taking into account the long-term and short-term effects of this intervention.

Keywords: Cytokines, Kidney Transplant, Acid-Base Balance, Clinical Trial

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Introduction

Nowadays kidney transplant as the main treatment for end-stage renal failure, has become one of the most common types of transplant and hence a big step in improving the life quality and expectancy. In the history of treatment, the life expectancy of post-transplant patients has greatly increased and the percentage of successful transplants continues to rise (1). From the beginning stages of kidney transplant therapy to now, there have been many changes in anesthesia techniques

and surgical methods as well as post-transplant aftercare, which have altogether helped improve longevity of the graft (2). Given the delicacy and importance of anesthesia as well as its important role in the success of transplant, there has been a growing body of literature on this treatment and any attempt to improve the quality of the anesthesia could play a major role in the survival of the patients (3). Patients with kidney failure suffer from chronic metabolic acidosis, hyponatremia, hypokalemia, and hyperchloraemia, which can potentially cause certain arrhythmias including bradycardia, heart block, hypotension, neuromuscular disorders as well as dyspnea and respiratory failure. In this regard, the control of acids and bases help prevent many potentially life-threatening disorders in these patients (4, 5). Given the importance of immunological factors in the transplant success or rejection, it is required to perform certain immunological compatibility experiments before transplantation, which is normally done in the transplant unit. The measurement of serum cytokines is helpful in evaluating the immunological status of the patient and assessing the effects of acidosis correction on immunological parameters. For example TNF- β , IFN- γ and IL-2 are important mediators of transplant rejection. IL-2 is essential in reproduction of T cells and production of efficient CTLs. IFN- γ plays the central role in the formation of delayed-type hypersensitivity response, macrophages infiltration into connective tissue, activation of them and conversion of them into destructive cells (6). The IL-2 and IFN-γ are among the cytokines reinforcing the cellular immune response, and their increased serum levels suggest poor prognosis of the transplantation. This is the fact while IL-10 represents an anti-inflammatory cytokine and inhibits the cellular immune response and, at the same time, increases the probability of a successful transplantation (7). The purpose of the present study was to evaluate the effect of acid-base balance on cytokines serum levels and short-term outcomes in kidney transplant recipients.

Methods

Study design

The present study was a randomized clinical trial with parallel design performed with a 1-1 ratio for the intervention and placebo group in Tehran Shahid Modarres Hospital (is the only governmental facility in north-western Tehran). The study population consisted of all patients with end-stage renal disease who were candidate for a kidney transplant from a living donor, had visited the kidney transplant unit of the hospital from late April in 2016 to early February in 2017, and had undergone a treatment with sodium bicarbonate (intervention group) or placebo (control group). The study participants included 40 patients with end-stage renal disease aged 18 to 70 who had undergone a kidney transplant from a living donor. We used a probabilistic sampling strategy for selection the included participants. To do this, a simple random sampling was considered by using random digit table.

The study inclusion criteria were: A. ASA Class I – II, B. Age range between 18 and 70 years and consent to participate in the study. The exclusion criteria were: A. Patients with end-stage cardiovascular problems, B. Metabolic acidosis with pH less than 7.15, C. Operation duration more than 3 hours, D. Body temperature less than 35 or greater than 38.5°C axilla before or during the operation, E. The need for blood transfusions during the operation, F. age over 70 or under 18 years, G. kidney transplant from a nonliving donor, H. a history of cancer and immunological diseases and immune modulatory medications.

Balanced block randomization techniques were used in the present study to randomly assign the participants in two intervention and control groups. Since blocking was done in 4 units in this study, a series of the random numbers 1 to 6 were produced using STATA to reach the desired sample size. Given that there are in total 6 states to arrange two subjects' in 4 blocks, if the number obtained were more than 6, the next number was produced without considering it. The sequences of balanced block randomization were obtained and put in sealed envelopes numbered with a 5-digit serial number by a third person who was not involved in the study design. All the envelopes (42 persons) had a random 5-digit serial number and were immediately opened after completion of the basic

information and tests of the subjects, allocating them to intervention group (receiving sodium bicarbonate) and placebo group (normal saline). The primary outcomes measured in this study were changes of serum cytokine such as IL-2, IL-10, IFN-γ and BUN and Cr serum after the treatment of acidosis in kidney transplant recipients. The baseline clinical and demographic data of the patient such as age, sex, weight, time elapsed since the last hemodialysis, start time of base ESRD and ABG, the ABG when opening the clamp and registration of TOF from the end of the induction to the end of operation were all entered in the patient's information form.

Treatment of metabolic acidosis

The study was conducted as follows: before induction of anesthesia, a catheter no.18 was implanted for fluids and medication. Invasive monitoring of blood pressure was performed under local anesthesia after Allen's test using an artery catheter no.20 through the radial artery of the nondominant hand. The same catheter was used to take arterial blood samples at the beginning of operation and on opening the clamps. First, the patients were preoxygenated with 100% oxygen for 3 minutes; then, based on a medical indication, midazolam with a dosage of 0.02 mg per kg and fentanyl 2μ/Kg were used. For induction of anesthesia, thiopental sodium with a dosage of 5 mg per kg was used. For muscle relaxation, atracurium with a dosage of 0.5 mg per kg was injected slowly. For maintenance of anesthesia, isoflurane with a dosage of 0.6-1% was used together with nitrous oxide at a ratio of 50%. During operation, if necessary, additional dosages of Atracurium were used. It is noteworthy that the last dose of Atracurium was administered while opening the clamp. After induction, central venous line was implanted through the right jugular vein. During the operation, the central venous pressure (CVP) was obtained at 10-12 cmH_2O and the patient's temperature was kept constant at 36°C and the patients in both groups were administered IV Lasix with the same dosage of 3 mg per kg and intravenous mannitol with a dosage of 0.5 g per kg. During this process, none of the patients included in the study required any blood infusion. Similarly, saline fluid at a rate of 50 ml per kilogram of body weight was used in all cadaver donors. Arterial blood samples of all the subjects were sent to the laboratory to analyze Arterial pH, arterial bicarbonate (HCO3), BE (Base Excess) and Pressure of Arterial carbon dioxide (PaCO2) after induction of anesthesia. Other blood samples were sent to the laboratory on opening the clamps. During the first week after transplantation, BUN, Cr and 24-hour urine volume were measured and the blood samples were taken and sent to immunology laboratory to check for the pre-induction immunologic markers before induction and once after opening the clamps. Blood samples and serum were taken from all the study participants at appropriate times (before correction of acidosis, after correction of acidosis and 7 days after operation) to measure the cytokines (IL-2, IL-10, IFN-γ).

The cytokines were measured using ELISA according to the instructions of the manufacturer's kit in the Laboratory of Department of Immunology, Shahid Beheshti University of Medical Sciences. A sample size of 21 patients in each group will be sufficient to detect a clinically important difference between the intervention and control group, using a two-tailed t-test of the difference between means, a power of 80%, and a significance level of 5%. The calculation is based on the assumption that the measurements on main variables are normally distributed.

ELISA assay

Levels of IFN γ, IL-2, and IL-10 in serum were measured using specific ELISA kits (Mabtech, Nacka Strand, Sweden), according to the manufacturer's instructions. All measurements were carried out in duplicates. OPD was used as chromogenic substrates for horseradish peroxidase (HRP). Color intensity in samples/standard wells was measured at 492 nm using an Anthos 2020 microplate reader (Anthos, Wals, Austria). Values of each protein of interest in a sample were calculated by extrapolation from standard curves generated in parallel with kit-provided standards. The level of sensitivity of the kits was 4-400 pg ml-1 for IFNγ, / IL-2 and 2-200 pg ml-1 for IL-10. Furthermore, to prevent the information bias due to measurement error, all the devices are calibrated regularly.

Statistical analysis

Continuous baseline demographic and clinical data are presented as mean ± standard deviation and grouped data as frequencies and percentages. Chi-square test or Fisher's exact test were used to determine the independence of the two categorical variables. Repeated measures of ANOVA were employed to investigate the mean differences of immune factors such as IL-2, IL-10 and IFN-γ between the groups over the time. To investigate the association between the treatment of metabolic acidosis and changes in serum Cr and BUN as well as immune factors such as IL-2, IL-10 and IFN-γ, the generalized estimation equation (GEE) method was undertaken. The GEE is a widely used estimation method for marginal (i.e., population-averaged) modeling of repeated data. In brief, GEEs use the generalized linear model to estimate more efficient and unbiased regression parameters relative to ordinary least squares regression, in part because they permit specification of a working correlation matrix that accounts for the form of within-subject correlation of responses on dependent variables of many different distributions, including normal, binomial, and Poisson (8). All analyses were done using STATA statistical software (version 13MP).

Ethical committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RAM.REC.1394.390;IRCT2015092712203N4) approved this study.

Results

In the present study, we used data from 40 ESRD patients candidate for receiving a kidney transplant from a living donor visiting Shahid Modarres Hospital in Tehran in 2016. As the figure below shows, finally and after excluding, in each group, 15 people failing to meet the inclusion criteria and 2 more ones who were lost to follow up, 20 patients were examined and analyzed.

In this study, the mean and standard deviation of age of the patients was 42±12.6 years. Of those examined, 29 ones (72.5%) were male and the rest were females (11 participants equivalent to 27.5%), respectively. The basic clinical information for the subjects in both groups is presented in Table 1.

The independent t-test determining the difference of the mean serum creatinine between the two groups shows that from the first to sixth day, there is no significant difference between the mean serum creatinine in the two groups and that the difference was observed only on the seventh day (P = 0.038).

In this study, generalized estimation equations models (GEE) was used to evaluate the effect of treatment on outcomes of creatinine and BUN concentrations in serum. The models are presented in Table 2.

The results showed that, the treatment of patient's acidosis did not affect serum creatinine levels. In other words, although the decrease in serum creatinine over time is statistically significant (p <0.001), this reduction is not attributable to the treatment of acidosis in kidney-transplant patients and there was no difference in the creatinine reduction between the intervention and control group. The results also show that correction of acidosis in kidney-transplant patients resulted in a significant reduction in BUN serum levels in the intervention group compared to the control group (Coefficient= -0.25, 95% CI:-0.5, -0.01). It should be noted, however, that the interaction effects between treatment and time were also significant and this means that treatment at different times would have different effects. Similarly, on investigating 24-hour urine volume within 7 days after transplantation in the intervention group (treatment of acidosis with bicarbonate) and control group (saline), the results of independent t-test showed that there was no statistically significant difference in average urine output between the control group (7870±478cc) and the intervention group (6973±445mL) (P =0.17). It should also be said that, following the investigation of the first-to-seventh-day urinary output in the two groups, the urine output was within the normal range in all the days except for the first day (due to intense hydration) and no statistical difference was observed (p> 0.05). Figure 2 shows the changes in the mentioned outcomes during 7 days after transplantation.

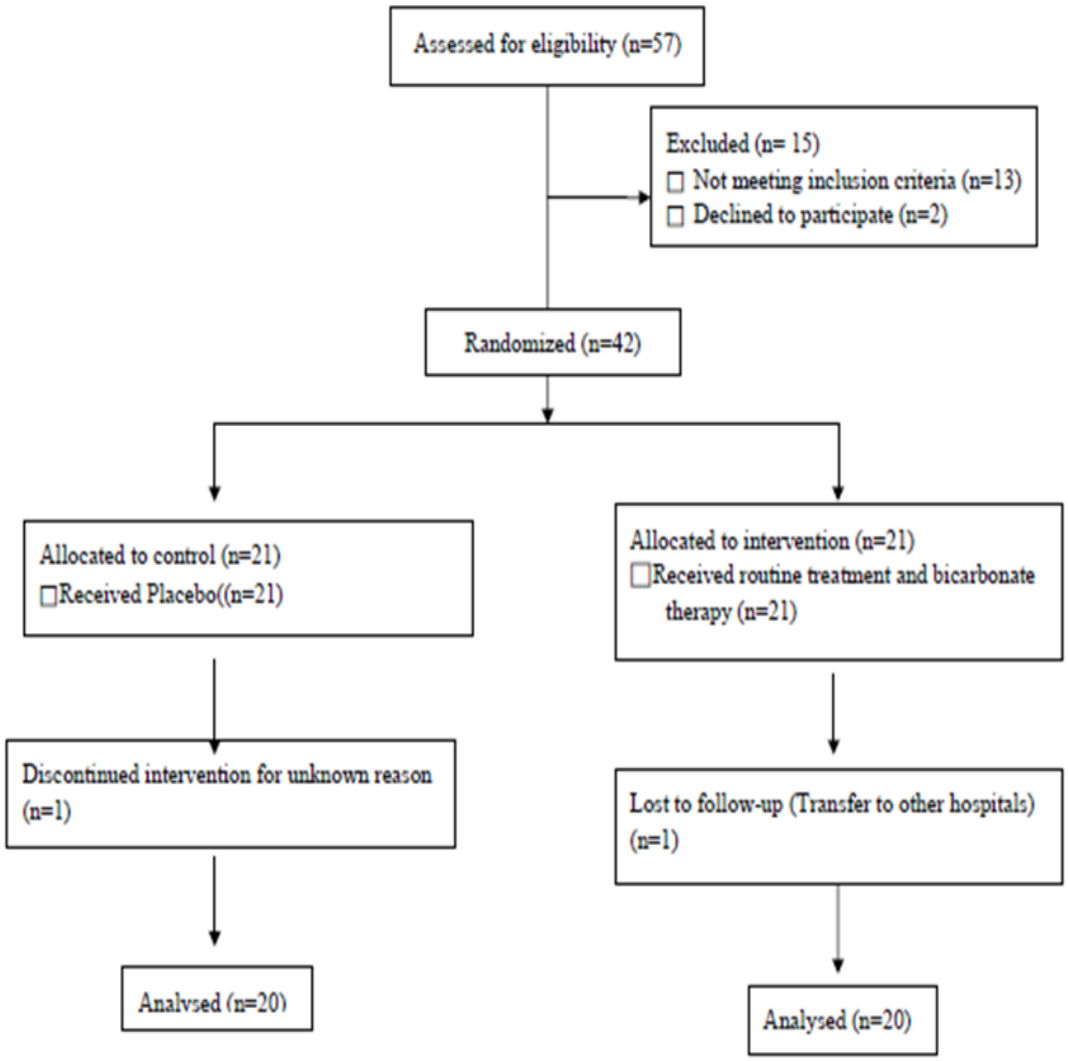

Figure 1. Flowchart of the studied participants.

The repeated measures of ANOVA showed that there is a significant difference in means of IL-2, IL-10, and IFN-γ between the intervention and control groups over the time (Table 3). Also, the mean changes of the factors under study over time and following the treatment of acidosis were statistically significant, in a way that with respect to the factors IFN-γ and IL-2, these changes were decremental in the intervention group and fairly incremental in the control group, while the changes of the factors IL-10 were contrary to those above. In all the models above, Mauchly's Test of Sphericity was meaningful; accordingly, Greenhouse-Geisser statistic was used.

The changes of the three factors examined are shown in Figure 3. In this figure, the time 1 represents that before the transplant, the time 2 represents the first day after the transplant and the time 3 represents the seventh day after the transplant.

For more tangible results in this study, GEE modeling approach was used to evaluate the effect of treatment on immunological factors. The results are given in Table 4. The results show that the time factor and its interaction with the type of treatment has a significant impact on the outcome under study and this means that the treatment has different effects in different times (the P interaction value is < 0.001 for all outcomes as shown in Table 4).

The table shows that correction of acidosis in

Table 1: Baseline characteristics and clinical data.

Baseline Variables	Intervention group	Control group
	Mean (SD)	Mean (SD)
Arterial PH	7.29±0.09	7.30±0.07
Arterial HCO3 (mmol/l)	16.4±4.1	16.6±2.71
Arterial PaCO2 (mmol/l)	32.3±7.3	30.1±3.73
Base excess (mEq/l)	-10.6±4.29	-8.35±3
BUN(mg/dl)	72.8±24.3	85±27.3
Cr(mg/dl)	3.54±1.48	3.9±1.56
IFN-γ(pg/ml)	8.41 (7.09-11.82)	9.14 (8.23-10.7)
IL-10 (pg/ml)	5.75 (3.51-9.17)	3.83 (2.71-7.84)
IL-2 (pg/ml)	7.34 (5.69- 7.88)	6.94(5.73-8.15)

Table 2: Results for the model of generalized equations structural analysis for investigation of the effects of acidosis treatment on Cr and BUN outcomes.

Outcomes	Variables	Coefficient,% 95 CI	P-value
Cr	Group	-0.009 (-0.26, 0.24)	0.93
	Time	-0.15 (-0.18, -0.12)	<0.001
	Interaction *	-0.015 (-0.05, -0.02)	0.42
BUN	Group	-0.25 (-0.5, -0.01)	0.041
	Time	-0.08 (-0.11, -0.05)	<0.001
	Interaction	0.049 (0.01, 0.08)	0.013

*Group time

Table 3: Results of repeated measures of ANOVA on evaluating the effects of acidosis treatment on outcomes IL-2, IL-10, IFN-γ.

Factors		Mean square	F	P value
IFN-γ	Group	91.59	6.29	0.017
	Factor	8.6	3.8	0.041
	Group * Factor	82.4	36.3	<0.001
IL-2	Group	56.84	4.9	0.033
	Factor	9.3	5.1	0.008
	Group * Factor	32.1	17.5	<0.001
IL-10	Group	245.01	12:54	0.001
	Factor	25.1	6.85	0.002
	Group * Factor	118.7	32.1	<0.001

patients reduces the IFN-γ by a factor of -1.74 in the intervention group compared to the group receiving saline (P=0.011). Also, the results showed that the levels of IL-2 was decreased after the treatment by a factors of -1.37 in intervention group compared to control group (p=0.025). The concentration of anti-inflammatory cytokine of IL-10 was incremental by a factor of 2.85 in intervention group compared to control group (P<0.001).

Discussion

The results of this study indicate that treatment of metabolic acidosis during the operation in patients undergoing kidney transplantation is effective in improving the outcome of transplant and the performance of kidney in these patients. In addition, it can enhanced in terms of functional factors, including BUN serum and the factors linked with the cellular immunity of the transplant, including the cytokines IL-2, IL-10, IFN-γ.

In the past two decades, the short-term outcomes of transplant have considerably improved,

Table 4: Results of GEE model on evaluating the effects of acidosis treatment on immunological factors.

Outcomes	Variables	Coefficient,% 95 CI	P-value
IFN-γ	Group	-1.74 (-3.09, -0.39)	0.011
	Time	-0.44 (-1.04, -0.03)	0.033
IL-2	Group	-1.37 (-2.57, -0.17)	0.025
	Time	-0.45 (-0.89, -0.004)	0.048
IL-10	Group	2.85 (1.29, 4.41)	<0.001
	Time	0.65 (0.01, 1.31)	0.044

but the corresponding long-term outcomes have hardly enhanced (9). Chronic renal transplant dysfunction is among the most common reasons leading to graft loss, which is evaluated by measuring kidney function over time. Several studies have been conducted to evaluate short-term and long-term performance of transplant. In these studies, the function of the kidneys is evaluated through clinical indices such as serum creatinine, glomerular filtration rate, and creatinine clearance. Moreover, the slope of the regression line of each of the above indices is determined, in terms of time, as the rate of progression in the transplant population and the patients with chronic kidney disease (10-12). In this study, to investigate the performance of transplant allograft within 7 days from the transplantation, the metabolic acidosis factor and its impact on the transplant outcome have been taken into account with emphasis on immune factors associated with transplant rejection besides the factors mentioned above.

Metabolic acidosis, occurring because of reduced serum concentrations of CO_2, can have adverse effects leading to rejection, depending on the patient's condition and the type of transplant. In a study conducted by Park et al, there was reported a risk of rejection following the increased serum concentrations of CO_2 at approximately 74 (13). In various studies, the prevalence of metabolic acidosis in kidney transplant patients was reported even up to 58 percent, and it is essential to address it considering the effects of this phenomenon on the outcome of kidney transplantation (14). Acidosis in kidney transplant patients is particularly important because, in addition to adverse effects on the hemodynamic system, it increases the production of cytokines and the activity of neutrophils in the body, which is ultimately detrimental to the transplant (15). Similarly, certain studies have addressed the role of post-transplant acidosis in mineral metabolism disorder in the body and its effect on the outcome of transplantation (14).

The present study suggests that short-term treatment of acidosis with bicarbonate sodium in the intervention group (compared to control group) leads to a reduction in BUN serum concentration with a coefficient of -0.25 within seven days after the transplantation, indicating the proper functioning allograft in this period. It should be noted that monitoring of metabolic acidosis in patients with ESRD should be done on a regular basis at least 1 year after transplantation. It important in order to maintain serum concentrations of CO_2 higher than 21 mEq/l, because the interaction of various factors such as metabolic acidosis caused by improper functioning of allograft, immune factors related to transplantation and the patient's condition may lead to acute rejection (16, 17). The results also suggest that treatment of acidosis does not significantly increase creatinine clearance in the intervention group compared to the control group and that both groups gradually witnessed an increase in clearance. However, in this study, reduced serum creatinine was not helpful in the treatment of metabolic acidosis, which is not the case for the studies by other researchers (18). The present study shows that the cytokines IFN-γ and interleukin 2 underwent a reduction after the treatment and correction of metabolic acidosis in patients while IL-10 began increasing. The study by Ori et al. evaluating the effects of correction of acidosis on cytokine secretion in patients with chronic kidney problem showed that the treatment of acidosis in CKD patients leads to decrease desecration of IL-10 but other chemokine including IL-1 β, IL-2, IL-6, TNF α, IFN γ, IL-1ra did not change significantly compared to before the transplant (19). In other study

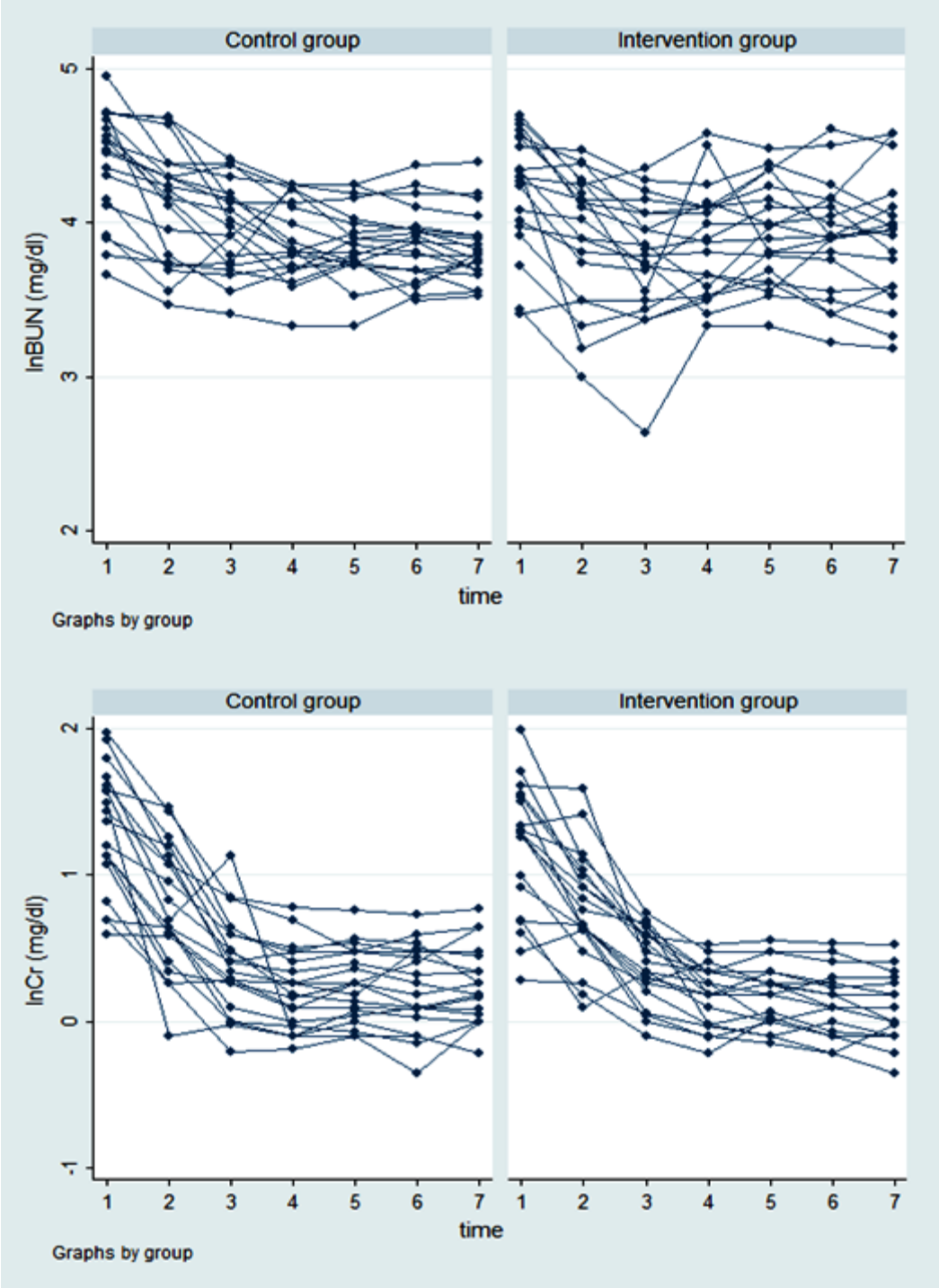

Figure 2. The changes of serum creatinine and BUN during seven days after kidney transplant.

on chronic antibody-mediated rejection and improper performance of allograft, it was shown that in this type of patients, the graft survival can greatly improve simply by regulating the IFNγ secretion (20).

The present study clearly demonstrates that treatment of metabolic acidosis in kidney transplant patients during anesthesia within seven days after transplantation leads to improved outcome of a transplant by reducing the secretion of IL-2 and Interferon Gamma. It should be noted, however, that the outcome of transplant is not affected by treatment of renal tubular acidosis alone and that

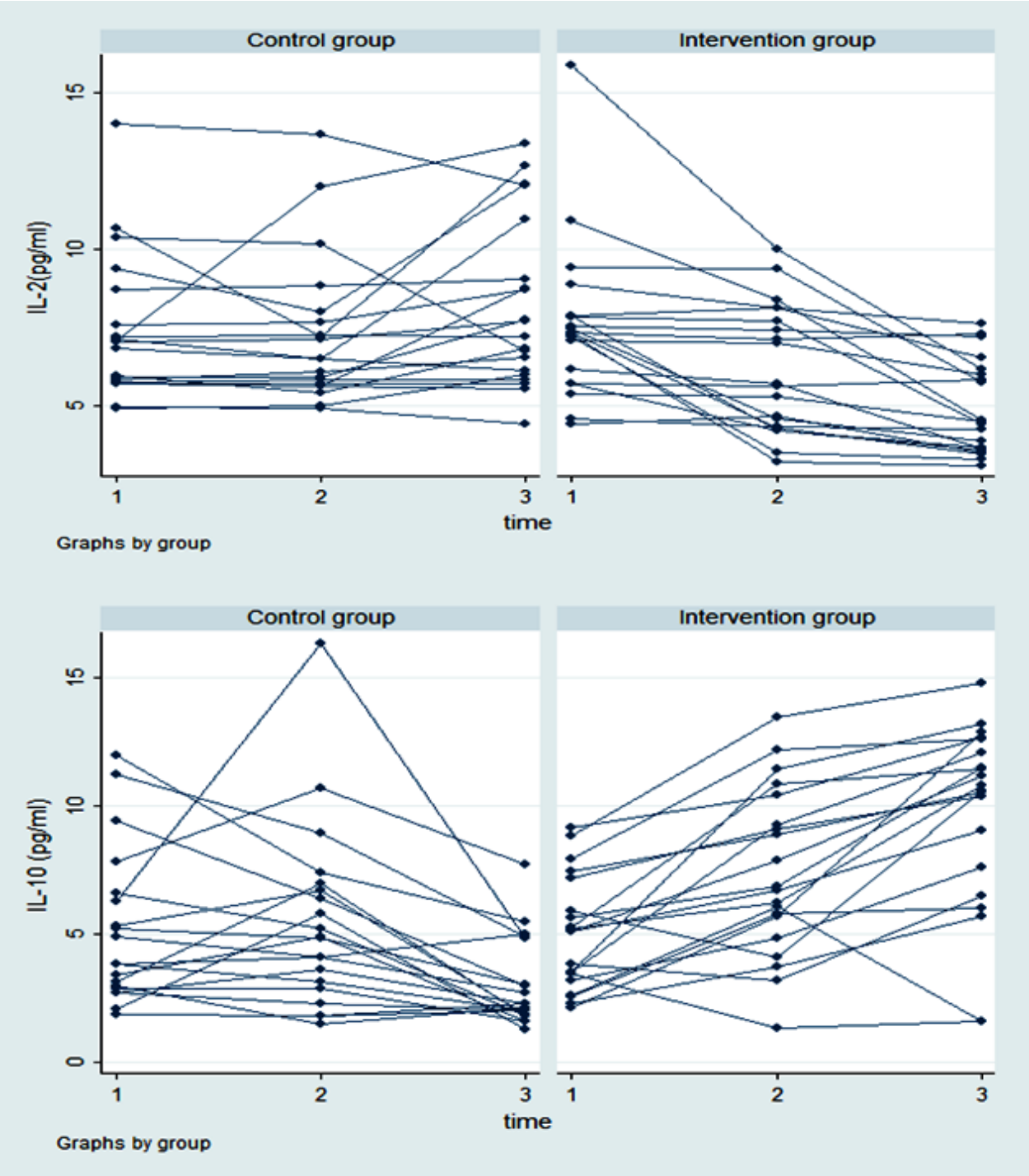

Figure 3. Change pattern of IL-2 and IL-10 during seven days after kidney transplantation.

immunosuppressive regimen, immune factors, personal characteristics and type of the graft are all effective in transplant rejection (17). For example, in a study on the effects of Interferon Gamma on the outcome, the researchers recommended that Pre-transplant determination of the numbers of IFN-γ-producing donor-specific memory cells be performed to predict acute rejection of transplanted cells and then the transplantation be carried out (21).

Despite the fact that metabolic acidosis is a common problem in kidney transplantation and can have negative effects, there is little information on

this topic (22). The first issue is lack of awareness about the exact prevalence of acidosis in kidney transplant patients and how it changes over time. The second issue is that there are not still extensive studies with sufficient sample size to make sure that the patients benefit from the correction of this disorder. The other issue is the uncertainty as to what extent of Acidosis should undergo treatment and what the ideal stage of this treatment is. Among the strengths of the present study is that it is the first to have addressed the effect of Acidosis correction on the outcome of kidney transplantation in patients. Although the results clearly suggest that correction of acidosis in renal transplant patients during surgery helps improve the performance of allograft in the short run, it is recommended that more extensive studies are carried out taking into account the long-term and short-term effects of this intervention and its cost-effectiveness for full investigation of the various aspects of correction of metabolic acidosis in kidney transplant patients.

Conclusion

Although the results clearly suggest that correction of acidosis in renal transplant patients during surgery helps improve the performance of allograft in the short run, but it is recommended that more studies be carried out taking into account the long-term and short-term effects of this intervention.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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