Metabolic acidosis in kidney transplant recipients

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KEY WORDS chronic kidney disease, kidney transplantation, metabolic acidosis

ABSTRACT

INTRODUCTION Metabolic acidosis (MA) may accelerate the progression of chronic kidney disease (CKD) and is an important risk factor for increased mortality in CKD patients. The clinical value of MA in kidney transplant (KTx) recipients has not been extensively studied so far.

OBJECTIVES The aim of this clinical single-center case-control study was to assess the prevalence of MA in KTx recipients in comparison with CKD patients and to identify pathogenic factors for MA in KTx recipients.

PATIENTS AND METHODS Venous blood concentrations of bicarbonate (HCO₃⁻) and blood hemoglobin concentrations were measured in 500 KTx recipients and 500 CKD patients matched for sex, age, and estimated glomerular filtration rate (eGFR). None of these patients received alkali treatment before the study. MA was diagnosed in KTx recipients with HCO₃⁻ levels lower than 22 mmol/l.

RESULTS The prevalence of MA was lower in KTx recipients than in CKD patients (12.0% vs 19.6%; P = 0.001). In both groups, the prevalence increased with progression of CKD stages (P <0.001 for trend) and was higher in patients with anemia. In a multivariable analysis, hemoglobin concentrations correlated independently with eGFR and HCO₃⁻ in KTx recipients (β = 0.314, P <0.001 and β = 0.274, P <0.001, respectively). Similar correlations were observed in CKD patients (β = 0.273, P <0.001 and β = 0.123, P = 0.006, respectively).

CONCLUSIONS Our study revealed that the prevalence of MA is lower in KTx recipients than in CKD patients. Moreover, in KTx recipients, blood bicarbonate concentrations are related to kidney function and blood hemoglobin concentrations.

INTRODUCTION Metabolic acidosis (MA) is a common consequence of chronic kidney disease (CKD). There are limited data about the prevalence of and pathogenic factors for MA in patients after kidney transplantation (KTx).¹ MA in CKD patients is mainly caused by insufficient production of bicarbonate (HCO₃⁻) in comparison with endogenous acid production and intake.²-⁵

MA adversely affects the quality of life and contributes to numerous systemic disorders in patients with CKD. It has been shown that MA is involved in the pathogenesis of malnutrition–inflammation–atherosclerosis syndrome by increased protein catabolism.⁶-⁷ Furthermore, a number of studies have shown that MA impairs calcium–phosphate homeostasis in this population.¹ MA reduces the sensitivity of the calcium receptor by lowering intracellular pH and thus stimulates the parathyroid glands to secrete parathyroid hormone. It was also observed that MA is associated with an increased risk of bone mass reduction in KTx recipients.⁸ Finally, it is well established that a decreased blood concentration of bicarbonate below 22 mmol/l is an independent risk factor for CKD progression.¹¹-¹⁴ Moreover, observational studies in CKD patients reported that MA is associated with a higher risk of mortality.¹⁵,¹⁶

Increased mortality was observed in patients with
blood concentrations of bicarbonate already below 23 mmol/l.16 The results of the above studies suggest that the target value of blood bicarbonate concentrations in patients with CKD should be higher than 22 to 23 mmol/l. The renoprotective properties of alkalinizing agents such as NaHCO₃, sodium citrate, and the so-called alkaline diet, have been shown in many clinical studies in CKD patients.17–24 To our knowledge, there is only one study assessing the impact of MA on mortality and long-term graft function in patients after KTx.25 Therefore, the aim of this study was to assess the prevalence of MA in KTx recipients in the late posttransplant period in comparison with CKD patients, and to analyze pathogenic factors related to MA in this population.

PATIENTS AND METHODS  The study group included 500 randomly selected patients at least 12 months after KTx (KTx in the years 1987–2010). The control group included 500 patients with CKD, matched for sex, age, and estimated glomerular filtration rate (eGFR) calculated with the abbreviated Modification of Diet in Renal Disease formula, which was validated in KTx recipients.24 None of the patients from either group received alkalinizing agents. All patients were treated in an outpatient clinic.

In all patients, the HCO₃⁻ concentration in venous blood was measured by the potentiometric method, using a GEM 3500 Premier analyzer (Werfen, Barcelona, Spain). MA was diagnosed when the HCO₃⁻ concentration was lower than 22 mmol/l.15–16 Diabetes mellitus was assessed on the basis of medical records. Proteinuria was detected using semiquantitative reagent strips with a sensitivity threshold of 30 mg/dl. Blood hemoglobin concentrations were determined by fluorescence flow cytometry (Sysmex XT-2000I, Sysmex Corporation, Kobe, Japan), while anemia was defined according to Kidney Disease Improving Global Outcomes guidelines: (hemoglobin <13 g/dl for men and hemoglobin <12 g/dl for women).27 The concentrations of immunosuppressive drugs, cyclosporine and tacrolimus, were measured in an accredited laboratory with an enzyme-linked immunosorbent assay. Blood samples were obtained before administration of the subsequent dose of drug.

In KTx recipients, the immunosuppressive regimen, treatment with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, as well as the presence of diabetes were assessed.

Statistical analysis was performed using Statistica 10.0 (StatSoft Inc, Tulsa, Oklahoma, United States). The Shapiro–Wilk test was used to assess the distribution of variables. Correlation coefficients were calculated according to the Spearman analysis. Differences between groups were tested with nonparametric analysis of variance and Mann–Whitney tests. In addition, a multivariable regression analysis was performed. The results were presented as means with SD or 95% CIs. Differences between groups were considered significant at a P-value level of less than 0.05.

RESULTS  There were 298 men (59.6%) in each group. In KTx recipients, the mean (SD) time since KTx was 76.2 (51.9) months. Most common causes of kidney disease in CKD patients were chronic glomerulonephritis (37.6%), arterial hypertension

<table>
<thead>
<tr>
<th>CKD stage</th>
<th>Characteristics</th>
<th>Sex, F/M, n</th>
<th>Age, y</th>
<th>eGFR, ml/min/1.73 m²</th>
<th>HCO₃⁻, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>All stages</td>
<td>CKD patients</td>
<td>29 (5.8)</td>
<td>13/16</td>
<td>42.9 (38.1–47.6)</td>
<td>111.48 (103.40–119.57)</td>
</tr>
<tr>
<td>All stages</td>
<td>KTx recipients</td>
<td>22 (4.4)</td>
<td>11/11</td>
<td>45.4 (39.33–51.47)</td>
<td>111.81 (98.52–125.11)</td>
</tr>
<tr>
<td>All stages</td>
<td></td>
<td>2 (12.6)</td>
<td>25(25.2)</td>
<td>48.4 (46.0–50.7)</td>
<td>73.37 (71.82–74.93)</td>
</tr>
<tr>
<td>All stages</td>
<td></td>
<td>3a (127.4)</td>
<td>43/84</td>
<td>48.4 (46.65–51.00)</td>
<td>51.2 (52.08–53.54)</td>
</tr>
<tr>
<td>All stages</td>
<td></td>
<td>3b (139.8)</td>
<td>61/78</td>
<td>51.2 (49.24–53.10)</td>
<td>37.78 (37.07–38.50)</td>
</tr>
<tr>
<td>All stages</td>
<td></td>
<td>4 (74.8)</td>
<td>3/41</td>
<td>52.2 (49.55–54.76)</td>
<td>23.72 (22.81–24.63)</td>
</tr>
<tr>
<td>All stages</td>
<td></td>
<td>5 (12.2)</td>
<td>4/5</td>
<td>48.7 (43.81–53.52)</td>
<td>12.78 (11.74–13.81)</td>
</tr>
</tbody>
</table>

Data are presented as mean (95% CI) unless otherwise indicated.

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; F, female; HCO₃⁻, bicarbonate; KTx, kidney transplant; M, male
(29.4%), diabetes (4.8%), and systemic lupus erythematosus (3.6%). In 11.8% of the patients, the cause of CKD was unknown. The details of the study population are presented in Table 1. Immunosuppressive agents and main comediations used in both groups are shown in Table 2.

In KTx recipients, the mean trough cyclosporine concentration in blood was 106.4 ng/ml (95% CI, 102.4–110.5) and the mean trough tacrolimus concentration was 7.1 ng/ml (95% CI, 6.8–7.4). The cyclosporine concentration was lower in patients with MA than in those without MA (95.4 ng/dl [95% CI, 86.9–103.8] vs 108.1 ng/dl [95% CI, 103.6–112.6], respectively; P = 0.02). Patients with and without MA had similar tacrolimus concentrations.

There was a slight difference in the mean HCO3⁻ levels between KTx recipients and CKD patients (25.38 mmol/l [95% CI, 25.09–25.67] vs 24.98 mmol/l [95% CI, 24.66–25.31], P = 0.056). In both groups, there was a trend for decreasing HCO3⁻ levels with progression of renal impairment (Figure 1). There were no significant differences in HCO3⁻ levels between men and women in any of the groups.

MA occurred less frequently in KTx recipients than in CKD patients (12.0% vs 19.6%, P = 0.001). In both groups, the incidence of MA increased with deteriorating kidney function (Figure 2). Additionally, positive correlations were found between eGFR and HCO3⁻ (for CKD, R = 0.443, P <0.001; for KTx recipients, R = 0.357, P <0.001). Of note, no significant correlations were found between age and HCO3⁻ in any of the groups. There were also no significant differences in the prevalence of MA between men and women in any of the groups.

Anemia was diagnosed in 28.8% of KTx recipients and 41.4% of CKD patients (P <0.001). The hemoglobin concentration was significantly higher in KTx recipients than in CKD patients (mean [95% CI], 13.4 g/dl [13.3–13.6] vs 12.8 g/dl [12.8–13.0], P <0.001). HCO3⁻ and eGFR were lower in patients with concomitant anemia than in those without anemia both in KTx recipients (mean [95% CI], 23.57 mmol/l

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**Table 2** Immunosuppressive agents and comediations used in kidney transplant (KTx) recipients and patients with chronic kidney disease (CKD)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>KTx recipients, n (%)</th>
<th>CKD patients, n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td>261 (52.2)</td>
<td>35 (7.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>223 (44.6)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycophenolate mofetil or mycophenolate sodium</td>
<td>393 (78.6)</td>
<td>17 (3.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>28 (5.6)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Everolimus or sirolimus</td>
<td>23 (4.6)</td>
<td>0 (0.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prednisone</td>
<td>235 (47.0)</td>
<td>181 (36.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metformin</td>
<td>14 (2.8)</td>
<td>33 (6.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erythropoiesis stimulating agents</td>
<td>26 (5.2)</td>
<td>14 (2.8)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Figure 1** Blood bicarbonate concentration at different stages of chronic kidney disease (CKD) in CKD patients and in kidney transplant (KTx) recipients. Blue boxes indicate means with 95% CIs. Abbreviations: ANOVA, analysis of variance.
KTx recipients treated and not treated with tacrolimus did not differ in HCO3⁻ levels (mean [95% CI], 25.35 mmol/l [24.88–25.83] and 25.40 mmol/l [25.04–25.76], respectively, \( P = 0.8 \)) or the prevalence of MA (11.6% vs 12.3%, respectively, \( P = 0.8 \)). Moreover, eGFR, HCO3⁻ levels, and the prevalence of MA in KTx recipients treated with cyclosporine were similar to those observed for patients treated with tacrolimus. There were no significant correlations between blood HCO3⁻ and trough levels of calcineurin inhibitors (cyclosporine or tacrolimus) in KTx recipients.

There were no significant differences in the prevalence of diabetes in KTx recipients treated and not treated with prednisone. However, HCO3⁻ level was higher in KTx recipients treated with prednisone (mean [95% CI], 25.7 mmol/l [25.3–26.1] vs 25.1 mmol/l [24.7–25.5]; \( P = 0.03 \)), while no differences were noted for eGFR or MA prevalence. Angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers were administered in 163 KTx recipients (32.6%), but there were no differences in HCO3⁻ levels or the prevalence of MA in patients treated and not treated with those drugs.

The multivariable analysis with the blood hemoglobin concentration as the dependent variable and eGFR and HCO3⁻ as independent variables showed that hemoglobin concentrations was correlated with eGFR and HCO3⁻ in KTx recipients (\( \beta = 0.314, P < 0.001 \) and \( \beta = 0.274, \) respectively, \( P < 0.001 \)) and CKD patients (\( \beta = 0.273, P < 0.001 \) and \( \beta = 0.123, \) respectively, \( P = 0.006 \)).

**DISCUSSION** Our paper presents the results from a single-center cross-sectional case-control study including 500 KTx recipients at least 1 year after KTx and 500 patients with CKD matched...
for age, sex, and eGFR. Considering the potential benefits of alkali therapy in patients after KTx, it is important to estimate the number of patients with MA and to identify its risk factors.

In our study, the prevalence of MA in KTx recipients was lower than in patients with CKD. As the study was descriptive, it was not possible to determine the causes of the lower prevalence of MA in KTx recipients than in patients with CKD. However, there are some possible explanations. Bicarbonate synthesis occurs in the tubulointerstitial compartment of the kidney. Kim et al. in an experimental study on mice, showed that renal denervation prevented interstitial inflammation (neutrophil and macrophage infiltration), reduced the number of myofibroblasts, and reduced the extent of interstitial fibrosis after a unilateral ureteral obstruction procedure. In another study, Kim et al. showed that renal denervation protected against the above abnormalities in mice exposed to kidney injury due to ischemia and reperfusion. Unlike native kidneys, transplanted kidneys are not innervated. Therefore, considering the results of the above studies, it can be speculated that tubulointerstitial damage is less pronounced in patients with transplanted noninnervated kidneys than in eGFR-matched patients with CKD and innervated native kidneys. Another possible line of evidence suggesting that MA is more specific to interstitial damage than to glomerular dysfunction is the high prevalence of MA in child and adolescent patients with CKD, in whom interstitial damage is more frequent than in adults with a similar eGFR. The observation that anemia due to insufficient erythropoietin synthesis by cells in the interstitium of the kidneys in KTx recipients is less frequent than in CKD patients also supports this hypothesis.

In adults, metabolic complications, including MA, occur mostly in stages 4 and 5 of CKD. Our results confirmed that the prevalence of MA in KTx recipients and CKD patients increases with the degree of kidney impairment, with the highest prevalence in CKD stage 5 (58.3% of KTx recipients and 62.5% of CKD patients) (Figure 2).

Clinical studies have shown that posttransplant anemia is diagnosed in 30% to 40% of patients. In the current study, the percentage of patients after KTx diagnosed with anemia was comparable and reached 28.8%. The Transplant European Survey on Anemia Management showed a strong association between blood hemoglobin concentrations and graft function impairment. In our study, MA was more frequent in patients with anemia both in KTx recipients and in patients with CKD. The multivariable analysis showed that in the posttransplant period the hemoglobin concentration was correlated with eGFR and HCO3⁻. Similar findings have been observed in other studies. Yorgin et al. showed a significant relationship between MA and anemia in KTx recipients. Due to the cross-sectional design of our study, we were not able to establish if anemia was the cause or consequence of MA.

Further interventional studies are needed to elucidate this issue.

In the current study, we demonstrated that MA occurred more often in patients with proteinuria. This finding may be explained by the fact that eGFR was significantly lower in KTx recipients with proteinuria than in those without proteinuria. It is assumed that the greater the severity of graft dysfunction in KTx recipients with proteinuria, the lower the HCO3⁻ levels and higher MA prevalence.

Previous studies have shown that the development of MA regardless of eGFR in patients with CKD depends on diabetes and younger age at disease onset (<65 years vs >65 years), among other factors. Long-term diabetes is associated with hyporeninemic hypoaldosteronism, tubular acidosis, and acidosis related to hypermetabolic state. Surprisingly, in the present study, diabetes did not affect the increase in the incidence of MA. However, these observations are consistent with the results of Caravaca et al., who showed that patients with stage 5 CKD and diabetes have less severe MA than those without diabetes.

Patients with CKD were recruited from a tertiary nephrology outpatient clinic; therefore, the distribution of the most common causes of CKD was different than that expected in the general CKD population (ie, diabetes only in 4.8% but glomerulonephritis in 37.6% of patients). Due to high prevalence of glomerulonephritis, the number of patients treated with steroids was closer to the number of KTx recipients. In KTx recipients and CKD patients treated with prednisone, blood bicarbonate concentrations were significantly higher than in those not receiving glucocorticoids. EGFR and the prevalence of MA were similar in both groups. It is assumed that the small increase in the bicarbonate concentration might be caused by the hypokalemic effect of glucocorticoids.

Renal tubular acidosis (RTA), which has been observed in previous studies in 13% to 17% of KTx recipients in early stages of kidney disease, may contribute to low bicarbonate concentrations. Due to the design of the current study, we were unable to determine the frequency of RTA in our cohort. Calcineurin inhibitor nephrotoxicity, acute and chronic rejection of the transplanted kidney, and ischemic tubular injury are known risk factors for RTA. In the 1980s, Stahl et al. observed a dose-dependent role of cyclosporine A in the pathogenesis of MA in patients after KTx. Mohbedi et al. elucidated the mechanism of action of tacrolimus on protein transport in the kidney, which plays a key role in the regulation of acid-base balance and causing MA. In one study, the decreased HCO3⁻ concentration was observed significantly more often in patients after KTx treated with cyclosporine and corticosteroids than with azathioprine. Another study reported that in patients in the late posttransplant period and with a eGFR exceeding 40 ml/min/1.73m², the incidence of RTA was higher in patients treated with
tacrolimus than with cyclosporine A.\(^4\) Considering the above findings, it could be speculated that chronic immunosuppressive therapy resulted in a higher prevalence of MA in KTx recipients than in patients with CKD. However, our results do not corroborate this hypothesis. In our study, most patients after KTx (96.8%) were treated with a calcineurin inhibitor. Therefore, the assessment of MA prevalence in untreated patients was impossible. Nevertheless, we showed that eGFR, blood bicarbonate concentration, and the prevalence of MA in KTx recipients did not depend on the type of calcineurin inhibitor. There were also no significant correlations between HCO\(_3\)- levels and blood concentrations of immunosuppressive agents in these patients.

Due to the risk of bleeding and local complications associated with arterial puncture as well as the need to spare arteries for the future access for hemodialysis, the acid-base balance parameters were measured only in venous blood, and not arterial blood, which seems to be an important limitation of the study. However, estimation of HCO\(_3\)- only in venous blood seems to be sufficient for a clinically useful diagnosis of MA.

Finally, considering that KTx recipients are characterized by a higher prevalence of MA and that alkali treatment has a renoprotective effect in patients with CKD, it seems reasonable to conduct further studies to clarify whether MA correction affects renal function and severity of anemia also in KTx recipients.

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**CONTRIBUTION STATEMENT** KS was involved data collection, statistical analysis, manuscript drafting, and literature review. DG was involved in statistical analysis, manuscript drafting, and literature review. MB was involved in data collection, statistical analysis, and manuscript drafting. MS was involved in statistical analysis and interpretation of results. AK was involved data collection and statistical analysis. LC was involved in interpretation of results. AW was responsible for planning the research, coordination and supervision of research, interpretation of results, and manuscript drafting. MA was responsible for planning the research, manuscript drafting, interpretation of results, and literature review. All authors edited and approved the final version of the manuscript.

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