Research Article

Posttransplantation Hypomagnesemia as a Predictor of Better Graft Function after Transplantation

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Keywords

Hypomagnesemia · Calcineurin inhibitors · Renal allograft function

Abstract

Background: Hypomagnesemia is frequently seen after transplantation and is particularly associated with the use of calcineurin inhibitors (CNIs). **Methods:** We conducted a retrospective, single-center analysis (2000–2013, $N = 726$) to explore the relationship between hypomagnesemia and long-term allograft outcome in kidney transplant recipients. For this study, a median serum magnesium (Mg) level of all measured Mg levels from 1 month to 1 year after renal transplantation was calculated. **Results:** For every increase in Mg by 0.1 mg/dL, the GFR decreased by 1.1 mL/min at 3 years posttransplant ($p < 0.01$) and by 1.5 mL/min at 5 years posttransplant. A median blood Mg level of ≥ 1.7 was found to be an independent predictor of a GFR < 60 mL/min at 3 years posttransplant. The odds of having a GFR < 60 mL/min 3 years posttransplant was almost 2-fold higher in the high Mg group than in the low Mg group. **Conclusions:** Hypomagnesemia from 1 to 12 months after renal transplantation is associated with a better allograft function up to 5 years posttransplant. This relationship was found to hold true after accounting for baseline allograft function and the presence of slow graft function.

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Introduction

Hypomagnesemia is a frequent complication in the posttransplant period and is particularly associated with the use of calcineurin inhibitors (CNIs) [1–3]. A lower serum magnesium (Mg) level and/or lower dietary Mg intake has been associated with an increased incidence of hypertension (HTN), metabolic syndrome, coronary artery disease, and ischemic stroke in previous studies [4–7]. In experimental animals, increased levels of extracellular Mg has been shown to cause vasorelaxation, decreased vascular resistance, and attenuate agonist-induced vasoconstriction [8–10], whereas decreased concentrations of Mg led to vasoconstriction, potentiated agonist-induced vasoreactivity, as well as increased vascular tone and blood pressure [11]. Furthermore, stroke patients intravenously infused with Mg had a significant vasodilatory response leading to a decrease in blood pressure [12]. Mg supplementation has also been shown to have anti-atherosclerotic effects [13]. Mg deficiency has been shown to cause endothelial cell dysfunction, inflammation, and oxidative stress, major contributors to atherosclerosis [9, 14], while Mg supplementation improved endothelial cell dysfunction in patients with coronary artery disease [15].

Additionally, an association between hypomagnesemia and impaired glucose metabolism has been demonstrated with hypomagnesemia being more frequently found in patients with type 2 diabetes [16] with serum Mg levels being inversely associated with glycemic control [17, 18]. Diabetes per se may induce hypomagnesemia, while higher Mg intake may confer a lower risk for type 2 diabetes [19, 20]. Separately, hypomagnesemia has been shown to decrease insulin sensitivity [21], indicating that hypomagnesemia is a potential cause of impaired glucose tolerance. Moreover, hypomagnesemia during the first month posttransplant was shown to be an independent predictor of new-onset diabetes following transplantation [22–24], a frequent complication which has been associated with adverse graft and cardiovascular outcomes [25, 26].

Hypomagnesemia is a common finding in CNI-treated patients and has been proposed as both a cause and a consequence of cyclosporine-induced nephrotoxicity. CNIs cause hypomagnesemia through suppressing reabsorption of Mg from renal tubules with Mg depletion thought to have a role in the development of HTN in renal transplant patients [28]. Hypomagnesemia has also been implicated as a contributing factor to the nephrotoxicity associated with cyclosporine. In an in vitro study of renal proximal epithelial cells, Mg supplementation attenuated cyclosporine-mediated cellular toxicity [29]. In an animal model of CsA nephrotoxicity, CsA-induced hypomagnesemia contributed to chronic renal fibrotic lesions seen during treatment through upregulation of fibrogenic molecules. Dietary supplementation of Mg ameliorated CsA-induced hypomagnesemia, almost completely abolished CsA-induced chronic fibrotic lesions, and improved glomerular dysfunction, at least in part, through inhibition of upregulated mRNA of endothelin-1 [30]. Mg supplementation significantly reduced interstitial fibrosis compared with CsA or the combination of CsA plus benazepril [31] and blunted CsA's fibrotic effects [32].

Despite this, it is still unknown whether low serum Mg levels affect disease progression and long-term allograft outcome in renal transplant recipients. Furthermore, most of the studies suggesting a poor renal outcome with hypomagnesemia in a transplant population were carried out in patients treated with cyclosporine [28–31]. The main objective of this study was to explore the relationship between hypomagnesemia and long-term allograft outcome in the kidney transplant population predominantly treated with tacrolimus. We also sought to assess the possibility of a relation between immunosuppression, hypomagnesemia, and allograft outcome.

Materials and Methods

Study Design

This is a retrospective analysis of data gathered from the Research Patient Data Registry system at our institution, in addition to data we collected from the clinical record. Biochemical data were retrieved in an automated fashion from the laboratory database and clinical data from electronic medical records. This study was approved by the local Ethics Committee.

Patients

All patients who underwent kidney transplantation at Brigham and Women's Hospital between January 2000 and September 2013 were evaluated for inclusion in the study and constituted a population of 856 transplants. Exclusion criteria were as follows: patients without serum Mg levels from 1 month to 1 year posttransplant ($n = 91$), including those whose graft failed in the first year posttransplant ($n = 39$).

Immunosuppression

Induction therapy consisted of either basiliximab (Simulect, Novartis Pharma, Basel, Switzerland) or thymoglobulin (Genzyme, Boston, MA, USA). All patients received corticosteroids, beginning with a single preoperative bolus of 500 mg of methylprednisolone intravenously, with gradual tapering to 20 mg of oral prednisone at postoperative day 5. If the patient was a candidate for early steroid withdrawal, the prednisone was continued at 20 mg PO daily until tacrolimus levels reached the therapeutic goal (>8 ng/mL) and then stopped. If the patient did not achieve therapeutic tacrolimus trough concentrations by postoperative day 12, the prednisone dose was tapered down below 20 mg/day. In patients who were not candidates for early steroid withdrawal, prednisone was tapered down to 5 mg/day within 2 months of transplant.

All patients were started orally on 1 g of mycophenolate mofetil (Cellcept, Roche Pharma, Basel, Switzerland) or 720 mg of mycophenolate sodium (Myfortic, Novartis Pharma) preoperatively; followed by 2×1 g/day or 2×720 mg/day, respectively, with tapering of the dosage depending on individual needs and/or side effects. CNi therapy was started posttransplantation, using mostly tacrolimus, aiming for trough levels of 8–10 ng/mL in the first 3 months and 5–8 ng/mL thereafter with reduction of trough goals if patients had side effects.

Biopsy-proven acute rejection was treated with 3 doses of intravenous methylprednisolone (500 mg) on consecutive days. All patients received *Pneumocystis jiroveci* pneumonia prophylaxis (sulfamethoprim cotrimoxazole or atovaquone). Prophylaxis for cytomegalovirus disease using ganciclovir (Cytovene, Roche Pharma) up to 2003 and then valganciclovir (Valcyte, Roche Pharma) was started in all patients.

Laboratory Data

The following biochemical posttransplantation variables were retrieved from the RPDR system: serum Cr, calcium, phosphate, intact parathyroid hormone (PTH), Mg, and tacrolimus 12-h trough blood levels. Median serum Mg values from 1 month to 1 year posttransplant were calculated using all samples available from this time period. We chose to look at serum Mg levels not earlier than 1 month posttransplant in order to prevent the confounding effect of delayed or slow graft function (SGF) and the early use of diuretics on blood Mg levels. Apart from the median Mg, the mean and the lowest registered values over this period were also recorded. As the mean values yielded similar results to median Mg, only the results based on the median will be reported here.

Presence or absence of SGF was determined based on whether serum Cr on postoperative day 5 was above 3 mg/dL (presence of SGF) or ≤ 3 (absence of SGF). We also retrieved intact PTH, calcium, and phosphate levels at 3 months posttransplant (or within the range of 2–4 months posttransplant, the closest value available to 3 months posttransplant) and at 1 year posttransplant (or within the range of 9–15 months posttransplant, the closest value available to 1 year posttransplant).

Median tacrolimus 12-h trough blood levels were calculated based on all trough levels available from 1 month to 1 year posttransplant in order to associate tacrolimus trough with serum Mg within the same time period. Both the median and mean trough levels of tacrolimus from 1 month to 1 year posttransplant were recorded. As those gave similar results, only the results based on the median are reported here.

Serum Cr levels were retrieved at different time points: 3 months posttransplant (3 months ± 2 weeks posttransplant), 1 year posttransplant (1 year ± 3 months posttransplant), 3 years posttransplant (3 years ± 3 months posttransplant), and 5 years posttransplant (± 6 months). Glomerular filtration rate was calculated according to the following CKD-Epi formula: $GFR = 141 \times \min(Scr/k, 1)^\alpha \times \max(Scr/k, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ (if female) $\times 1.159$ (if black) ($K = 0.7$ if female, 0.9 if male; $\alpha = -0.329$ if female, -0.411 if male; min = the minimum of Scr/k or 1; max = the maximum of Scr/k or 1).

Study Assessments

Based on the electronic patient medical records, details on the following relevant items were collected: age, gender, race, transplant type, type of induction treatment, medical history (specifically history of HTN, heart failure [HF], or cardiovascular disease [CVD]), rejection episodes from 1 month to 1 year posttransplant, and use of any loop diuretics from 1 month to 1 year posttransplant. The nature of the original kidney disease was noted as either “diabetic nephropathy” or “others.”

Statistical Analysis

Descriptive statistics were expressed as percentages for categorical data or mean ($\pm$ SD) for continuous variables. Differences in baseline characteristics between the groups were tested using χ^2 for the categorical variables or t test for the continuous variables. We analyzed subgroups based on the availability of serum Cr at 1, 3, and 5 years posttransplant.

A multivariable linear regression analysis was constructed to analyze the association between median Mg levels and allograft outcome, while adjusting for the following potential confounders: age, race, gender, transplant type, induction therapy, medical history of HTN, HF and CVD, median tacrolimus 12-h trough levels, rejection episodes, use of loop diuretics, ESRD secondary to diabetes, presence of SGF, and baseline allograft function which was derived from the calculated GFR at 3 months posttransplant. The variables used in the multivariate analysis were chosen based on their clinical relevance, that is, those with possible interaction with blood Mg levels and/or influence on allograft function. A multivariable logistic regression model was constructed to assess the independent risk factors associated with low GFR (<60 mL/min).

In order to account for patient-level variability, a repeated-measures analysis was performed using a linear mixed-effects model with a random intercept calculated for each patient ID number. The median Mg level, years posttransplant, and the aforementioned confounders were used as the fixed effects. p values are provided via Satterthwaite’s degrees of freedom method. The mixed-effects model analysis was performed using the R packages *lme4* and *lmerTest*.

A p value of less than 0.05 was considered statistically significant. Data were analyzed using R version 3.5.2 (<http://www.r-project.org>).

Table 1. Patient characteristics

	Mg < 1.7 (N = 407)	Mg ≥ 1.7 (N = 319)	p value
Mg, N	15.24 (11.18)	21.76 (23.93)	<0.001
Maximum Mg	1.82 (0.3)	2.14 (0.33)	<0.001
Minimum Mg	1.27 (0.18)	1.49 (0.23)	<0.001
Age, years	50.7 (13.6)	51.9 (13.4)	0.212
Gender			
Males	220 (54.1)	185 (58.0)	0.324
Females	187 (45.9)	134 (42.0)	
Race/ethnicity			
White	261 (64.1)	196 (61.4)	0.402
Black	79 (19.4)	71 (22.3)	
Asian	25 (6.1)	14 (4.4)	
Hispanic	24 (5.9)	27 (8.5)	
Other	18 (4.4)	11 (3.4)	
ESRD d/t diabetes	46 (11.3)	54 (16.9)	0.038
Transplant type			
LRD/LURD	228 (56.0)	173 (54.2)	0.392
SCD	101 (24.8)	72 (22.6)	
DCD/ECD	78 (19.2)	74 (23.2)	
Type of induction			
Thymoglobulin	277 (68.1)	202 (63.3)	0.562
Simulect	97 (23.8)	87 (27.3)	
None	31 (7.6)	29 (9.1)	
CNI treatment	407 (100%)	308 (96.6%)	0.001
TACRO_median, ng/mL	8.81 (1.76)	7.64 (1.84)	<0.001
Rejection episodes per patient	0.04 (0.21)	0.03 (0.19)	0.494
Medical history			
HTN	349 (85.7)	272 (85.3)	0.938
CVD	209 (51.4)	173 (54.2)	0.486
HF	151 (37.1)	130 (40.8)	0.355
Use of diuretics			
PO loop diuretics	64 (15.7)	81 (25.4)	0.002
IV loop diuretics	32 (7.9)	55 (17.2)	<0.001
SGF	131 (32.2)	131 (41.1)	0.017
Laboratory tests			
CAL_3M, mg/dL	9.68 (0.75)	9.63 (0.74)	0.428
CAL_1Y, mg/dL	9.64 (0.68)	9.57 (0.81)	0.222
PHO_3M, mg/dL	2.52 (0.74)	2.75 (0.79)	<0.001
PHO_1Y, mg/dL	2.75 (0.68)	2.98 (0.82)	<0.001
PTH_3M, ng/L	158.21 (146.75)	152.38 (235.68)	0.804
PTH_1Y, ng/L	156.84 (182.21)	169.00 (216.61)	0.605
GFR_3M, mL/min	53.20 (18.03)	48.67 (18.53)	0.002

Mg, magnesium; CNI, calcineurin inhibitor; TACRO, tacrolimus; HTN, hypertension; CVD, cardiovascular disease; HF, heart failure; SGF, slow graft function; CAL, calcium; PHO, phosphate; PTH, parathyroid hormone.

Results

Seven hundred twenty-six transplants (405 men and 321 women) of the original population of 856 transplants fulfilled the inclusion criteria. Of these, 407 (56%) had a median Mg level from 1 month to 1 year posttransplant below 1.7 (low Mg group) and 319 (44%) had a median Mg level equal to or above 1.7 (high Mg group).

Table 2. Graft function in the low Mg and high Mg groups

	Mg < 1.7	N	Mg ≥ 1.7	N	p value
GFR 1 year, mL/min	55.25 (19.19)	348	49.24 (19.53)	273	<0.001
GFR 3 years, mL/min	52.30 (21.78)	286	45.80 (19.28)	193	0.002
GFR 5 years, mL/min	50.09 (24.47)	208	44.99 (21.93)	100	0.07
Mg, magnesium.					

Univariate Comparison of Low Mg versus High Mg Groups

Pre- and posttransplantation clinical and biochemical parameters comparing the low and high Mg groups are presented in Table 1. Mean age was 50.7 ± 13.6 in the low Mg group and 51.9 ± 13.4 in the high Mg group. A total of 11.3% of the patients in the low Mg group and 16.9% in the high Mg group had ESRD secondary to diabetes.

All patients with low Mg were treated with CNIs versus 96.6% of the patients in the high Mg group ($p = 0.001$). Patients with low Mg had higher median and mean tacrolimus blood levels from 1 month to 1 year posttransplant ($p < 0.001$) and lower phosphate levels at 3 months ($p < 0.001$) and 1 year ($p < 0.001$) posttransplant than patients with high Mg (see Table 1). There were more rejection episodes from 1 month to 1 year posttransplant in the low Mg group than in the high Mg group, but the difference between the groups was not statistically significant. Medical history of HTN, HF, or CVD was also not significantly different between the groups. Due to the association of loop diuretics with increased urinary Mg excretion [33], we looked at the use of loop diuretics from 1 month to 1 year posttransplant. The use of loop diuretics was higher in the high Mg group than in the low Mg group (25.4% compared to 15.7% of the patients used PO loop diuretics [$p < 0.002$] and 17.2% compared to 7.9% of the patients were treated with intravenous loop diuretics [$p < 0.001$]). A total of 32.2% of the patients in the low Mg group had SGF compared to 41.1% in the high Mg group ($p = 0.017$). Baseline allograft function derived from GFR at 3 months posttransplant was higher in the low Mg group than in the high Mg group (53 ± 18 vs. 48.7 ± 18.5 ; $p = 0.002$). Other nonsignificant differences between variables in the 2 groups were the prevalence of ESRD attributed to diabetes, transplant type, type of induction, and blood Ca and PTH levels.

Univariate Outcome Comparison of Low Mg versus High Mg

GFR was found to be significantly higher in the low Mg group than in the high Mg group at 1 year (55.2 ± 19.2 vs. 49.2 ± 19.5 ; $p < 0.001$), 3 years (52.3 ± 21.8 vs. 45.8 ± 19.3 ; $p = 0.002$), and 5 years posttransplant (50 ± 24.5 vs. 45 ± 21.9 ; $p = 0.07$) (Table 2; Fig. 1).

Linear Regression Analysis with Mg and GFR as Continuous Variables

We performed a multivariable linear regression analysis to establish the change in GFR in relation to blood Mg level. For every increase in Mg by 0.1 mg/dL, GFR decreased by 1.1 mL/min at 3 years posttransplant (CI 0.3–1.96; $p < 0.01$) and by 1.5 mL/min at 5 years posttransplant (CI 0.09–2.84; $p < 0.01$). For every increase in baseline allograft function by 1 mL/min, GFR increased by 0.8 [0.7–0.9], 0.6 [0.5–0.7], and 0.6 [0.45–0.78] mL/min at 1, 3, and 5 years posttransplant, respectively ($p < 0.01$).

Black race was associated with a worse graft function at 5 years posttransplant. Compared to whites, GFR decreased in blacks by 7.5 [0.8–14.2] mL/min at 5 years posttransplant ($p < 0.01$). This is in compliance with previous publications demonstrating worse renal allograft outcomes in blacks [34–36]. For every rejection episode, GFR at 1 year posttransplant decreased by 5.9 [10.9–0.9] mL/min ($p < 0.01$). Rejections from 1 month to 1 year posttrans-

Fig. 1. Graft function post renal transplantation in the low Mg and high Mg groups. Mg, magnesium.

plant were not found to be associated with allograft function at 3 and 5 years posttransplant. Use of loop diuretics from 1 month to 1 year posttransplant was found to be associated with reduced GFR at 1 year posttransplant but had no significant association with allograft outcome at 3 and 5 years posttransplant. Presence of HF was found to be an independent predictor of reduced allograft function at 5 years posttransplant ($p < 0.01$), while CVD was found to be an independent predictor of a worse graft function at 1, 3, and 5 years posttransplant ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively). All other variables including age, gender, transplant type, induction therapy, ESRD secondary to diabetes, median CNI trough level, and the presence of SGF were not significantly associated with differences in GFR at 1, 3, and 5 years posttransplant (Table 3).

Logistic Regression Analysis for GFR <60 mL/min

We performed a multivariate logistic regression analysis to identify predictors of GFR below 60 mL/min at 1, 3, and 5 years posttransplant. Not surprisingly, baseline allograft function was found to be an independent predictor of GFR <60 mL/min at 1, 3, and 5 years posttransplant. Median blood Mg level from 1 month to 1 year posttransplant equal to or above 1.7 was found to be an independent predictor of GFR <60 mL/min at 3 years posttransplant as the odds for GFR <60 mL/min was almost 2-fold higher in the high Mg group than in the low Mg group after adjusting for baseline allograft function (CI 1.02–3.7; $p < 0.05$). It is possible we could not show significant differences for the odds of GFR <60 mL/min in the high Mg group compared to the low Mg group at 1 and 5 years posttransplant as the Mg and GFR cutoffs are too arbitrary. The odds for GFR <60 mL/min at 5 years posttransplant were 3.6 times higher in patients with HF than in patients without HF [1.5–8.6] ($p < 0.01$). The presence of CVD significantly increased the odds for GFR <60 mL/min at 1 and 3 years posttransplant

Table 3. Change in GFR in relation to blood Mg levels using multivariate linear regression model

	GFR 1 year post Tx, study population (n = 542), mean (SD)	GFR 3 years post Tx, study population (n = 415), mean (SD)	GFR 5 years post Tx, study population (n = 273), mean (SD)
Increase in Mg by 0.1, mg/dL	−0.46 (−0.97, 0.05)	−1.12 (−1.96, −0.3)**	−1.5 (−2.84, −0.09)*
Age (increase in 1 year)	−0.0 (−0.09, 0.07)	−0.1 (−0.25, 0.03)	0.0 (−0.2, 0.2)
Gender (female vs. male)	0.3 (−1.65, 2.3)	−0.23 (−3.7, 3.26)	0.92 (−4.2, 6.0)
Race			
White	Reference	Reference	Reference
Black	−0.6 (−3.2, 1.9)	−2.2 (−6.8, 2.34)	−7.5 (−14.2, −0.8)*
Asian	0.71 (−3.2, 4.6)	−1.5 (−8.2, 5.15)	−3.3 (−13.23, 6.7)
Hispanic	−1.6 (−5.3, 2.05)	−2.9 (−9.1, 3.31)	−6.2 (−15.8, 3.4)
Other	−0.54 (−6.26, 5.2)	−2.5 (−13.7, 8.7)	−6.6 (−21.1, 7.9)
Transplant type			
DCD/ECD versus LRD/LURD	−0.2 (−3.8, 2.9)	1.2 (−4.1, 6.6)	2.9 (−5.5, 11.5)
SCD versus LRD/LURD	−0.35 (−3.0, 2.3)	−0.7 (−5.1, 3.8)	2.74 (−3.8, 9.2)
Induction			
Thymoglobulin	Reference	Reference	Reference
Simulect	0.37 (−1.8, 2.5)	1.8 (−2.01, 5.7)	0.6 (−5.7, 6.9)
No induction	−3.6 (−7.5, 0.3)	−4.0 (−10.4, 2.4)	2.0 (−6.7, 10.7)
Medical history			
HTN	−1.3 (−4.3, 1.7)	2.2 (−3.35, 7.7)	−4.7 (−12.1, 2.71)
HF	−0.9 (−3.14, 1.29)	−3.2 (−7.1, 0.7)	−8.41 (−14.1, −2.7)**
CVD	−3.28 (−5.3, −1.2)**	−5.5 (−9.2, −1.9)**	−5.5 (−10.7, −0.3)*
Median CNI trough level	0.05 (−0.5, 0.6)	−0.0 (−0.98, 0.98)	0.2 (−1.2, 1.5)
Rejection episodes	−5.9 (−10.9, −0.9)**	−6.34 (−14.8, 2.1)	−8.4 (−19.5, 2.8)
Use of loop diuretics	−4.98 (−7.6, −2.4)**	−1.9 (−6.5, 2.7)	2.2 (−4.9, 9.2)
ESRD d/t diabetes versus other etiologies	−1.5 (−4.3, 1.3)	−1.2 (−6.0, 3.5)	−2.6 (−9.5, 4.2)
SGF versus no SGF	0.41 (−2.01, 2.8)	−2.5 (−6.7, 1.6)	0.5 (−5.7, 6.7)
GFR_3M increase in 1, mL/min	0.8 (0.7, 0.9)**	0.6 (0.5, 0.7)**	0.6 (0.45, 0.8)**

The results shown in this table were derived from 4 separate linear regression models; each of them adjusted for the following covariates: age, race, gender, transplant type, induction therapy, medical history, median CNI, trough level, rejection episodes, use of diuretics, ESRD, secondary to diabetes, presence of SGF, and baseline GFR. Mg, magnesium; HTN, hypertension; HF, heart failure; CVD, cardiovascular disease; CNI, calcineurin inhibitor; SGF, slow graft function. * $p < 0.05$. ** $p < 0.01$.

($p < 0.001$ and $p < 0.05$, respectively). All other variables, including age, gender, race, transplant type, induction therapy, ESRD secondary to diabetes, median CNI trough level, rejection episodes, use of loop diuretics, history of HTN, and the presence of SGF, were not significantly associated with GFR below 60 mL/min at 1, 3, and 5 years posttransplant (Table 4).

Mixed-Effects Model

In order to analyze all the collected measures together while accounting for patient-level measurement variability and dependence, a repeated-measures analysis was performed using a linear mixed-effects model. In this analysis, GFR was found to be associated with time posttransplant (every 1 year posttransplant, GFR decreased by 1.5 mL/min; CI 1.6–1.4; $p < 0.01$), median Mg (for every increase in median Mg by 0.1 mg/dL GFR decreased by 1.1 mL/min; CI 0.4–1.8; $p < 0.01$), medical history of HF (GFR decreased by 4.3 mL/min in renal transplant recipients with HF compared those without HF; CI 1.13–7.6; $p < 0.01$), CVD (GFR decreased by 5.4 mL/min in renal transplant recipients with CVD compared those without CVD; CI 2.4–8.4; $p < 0.01$), and baseline allograft function (GFR increased by 0.65 mL/min for every increase in baseline GFR by 1 mL/min; CI 0.6–0.7; $p < 0.01$). All other variables including gender, race, transplant type, induction therapy, ESRD secondary to diabetes, median CNI

Table 4. Odds of GFR below 60 mL/min using multivariate logistic regression model

	GFR 1 year post Tx, study population (n = 542), mean (SD)	GFR 3 years post Tx, study population (n = 415), mean (SD)	GFR 5 years post Tx, study population (n = 273), mean (SD)
High versus low Mg	1.3 (0.7, 2.4)	1.94 (1.02, 3.7)*	0.97 (0.4, 2.2)
Age (increase in 1 year)	1.0 (0.97, 1.02)	1.02 (1.0, 1.04)	1.0 (0.97, 1.03)
Gender (female vs. male)	1.15 (0.66, 2.0)	0.8 (0.42, 1.4)	1.02 (0.5, 2.1)
Race			
White	Reference	Reference	Reference
Black	1.0 (0.4, 2.3)	1.5 (0.64, 3.7)	2.3 (0.8, 7.5)
Asian	0.9 (0.3, 2.7)	0.9 (0.3, 2.8)	3.2 (0.8, 14.8)
Hispanic	1.8 (0.7, 5.6)	1.5 (0.5, 4.5)	1.3 (0.4, 4.6)
Other	1.9 (0.3, 13.8)	9.6 (1.06, 231)	4.75 (0.6, 105)
Transplant type			
DCD/ECD versus LRD/LURD	1.17 (0.4, 3.5)	1.3 (0.4, 3.7)	0.9 (0.24, 3.6)
SCD versus LRD/LURD	1.03 (0.5, 2.2)	0.93 (0.4, 2.0)	0.7 (0.3, 1.7)
Induction			
Thymoglobulin	Reference	Reference	Reference
Simulect	1.0 (0.6, 1.9)	0.9 (0.4, 1.7)	1.25 (0.5, 2.9)
No induction	1.1 (0.4, 3.2)	2.3 (0.8, 8.0)	1.14 (0.4, 3.7)
Medical history			
HTN	1.8 (0.9, 3.8)	0.8 (0.3, 1.9)	2.04 (0.8, 5.0)
HF	1.1 (0.6, 2.2)	1.7 (0.84, 3.53)	3.6 (1.5, 8.6)**
CVD	2.8 (1.6, 5.0)**	2.06 (1.1, 3.9)*	1.8 (0.9, 3.6)
Median CNI trough level	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	0.9 (0.7, 1.0)
Rejection episodes	2.7 (0.6, 11.5)	3.8 (0.9, 18.5)	1.8 (0.4, 8.1)
Use of loop diuretics	1.43 (0.63, 3.42)	1.0 (0.4, 2.6)	0.5 (0.2, 1.6)
ESRD d/t diabetes versus other etiologies	1.19 (0.5, 2.8)	1.2 (0.5, 3.0)	2.4 (0.85, 7.7)
SGF versus no SGF	0.9 (0.4, 2.0)	0.56 (0.3, 1.2)	0.85 (0.3, 2.2)
GFR_3M increase in 1 mL/min	0.87 (0.84, 0.9)**	0.9 (0.9, 0.93)**	0.92 (0.9, 0.94)**

The results shown in this table were derived from 4 separate linear regression models; each of them adjusted for the following covariates: age, race, gender, transplant type, induction therapy, medical history, median CNI, trough level, rejection episodes, use of diuretics, ESRD, secondary to diabetes, presence of SGF, and baseline GFR. Mg, magnesium; HTN, hypertension; HF, heart failure; CVD, cardiovascular disease; CNI, calcineurin inhibitor; SGF, slow graft function. * $p < 0.05$. ** $p < 0.01$.

trough level, rejection episodes, history of HTN, use of loop diuretics, and the presence of SGF were not significantly associated with GFR over time (Table 5). The variation in GFR between patients was estimated to be 11.2 mL/min.

Discussion

We demonstrated that hypomagnesemia from 1 to 12 months posttransplant is independently associated with a better renal graft function at 3 and 5 years posttransplant after adjustments in a multivariate analysis to baseline allograft function and to the presence of SGF. This strengthens the independent association of hypomagnesemia with allograft outcome even though lower Mg levels may be a surrogate for a better GFR. To our knowledge, this is the first study on transplant population showing the relationship between blood Mg levels early posttransplant and long-term graft function, despite the high incidence of hypomagnesemia among transplant recipients.

Moreover, we demonstrated that the association between the use of CNI on the one hand, which is strongly related to hypomagnesemia, and renal graft function on the other hand

Table 5. Mixed-effects model

	GFR, mean (SD)
Years posttransplant	–1.5 (–1.6, –1.4)**
Increase in Mg by 0.1, mg/dL	–1.13 (–1.8, –0.4)**
Gender (female vs. male)	–0.12 (–2.9, 2.74)
Race	
White	Reference
Black	–1.9 (–5.7, 1.8)
Asian	–1.6 (–7.3, 4.1)
Hispanic	–4.2 (–9.5, 1.1)
Other	–6.5 (–14.8, 1.9)
Transplant type	
DCD/ECD versus LRD/LURD	–0.85 (–5.3, 3.6)
SCD versus LRD/LURD	–0.0 (–3.8, 3.8)
Induction	
Thymoglobulin	Reference
Simulect	–0.7 (–3.9, 2.5)
No induction	1.15 (–4.6, 6.9)
Medical history	
HTN	–3.1 (–7.6, 1.3)
HF	–4.3 (–7.6, –1.13)**
CVD	–5.4 (–8.4, –2.4)**
Median CNI trough level	–0.26 (–1.09, 0.58)
Rejection episodes	–6.9 (–14.29, 0.49)
Use of loop diuretics	–3.0 (–6.7, 0.7)
ESRD d/t diabetes versus other etiologies	–2.2 (–6.14, 1.8)
SGF versus no SGF	0.94 (–2.6, 4.4)
GFR_3M increase in 1 mL/min	0.65 (0.6, 0.7)**

The results shown in this table were derived from 4 separate linear regression models; each of them adjusted for the following covariates: age, race, gender, transplant type, induction therapy, medical history, median CNI, trough level, rejection episodes, use of diuretics, ESRD, secondary to diabetes, presence of SGF, and baseline GFR. Mg, magnesium; HTN, hypertension; HF, heart failure; CVD, cardiovascular disease; CNI, calcineurin inhibitor; SGF, slow graft function. ** $p < 0.01$.

disappeared after adjustment for Mg levels in a multivariate model (Tables 3–5). We included in the model rejection episodes to adjust for possible effect of lower CNI trough levels associated with higher Mg levels on allograft outcome by increased rejection rate. Both CNI trough levels and rejection rate were not found to be associated with allograft outcome in the multivariate analysis. This suggests that the effect of CNI on renal graft function, at least in part, is related to hypomagnesemia.

The precise mechanism by which CNIs provoke Mg wasting is not clear. Approximately 60% of the filtered Mg is reabsorbed in the loop of Henle and another 5% in the distal tubule [33]. Although a relatively small amount of Mg is reabsorbed in the distal tubule, the amount accounts for about 60% of the total amount of Mg delivered to this segment. Therefore, the loop of Henle and/or distal tubule could likely be candidates for sites where CNIs work to inhibit renal Mg reabsorption irrespective of the glomerular filtration rate [37]. This might be due to obligatory renal loss mediated by CNI-induced reduced renal expression of the transient receptor potential melastatin 6, the Mg transporter in the distal collecting tubule [27].

We looked specifically at the association of tacrolimus-induced hypomagnesemia to graft function, while nearly all of the data on CNI toxicity are related to CsA [28–31] rather than

tacrolimus. Moreover, tacrolimus had a more profound effect on Mg metabolism than CsA early after hematopoietic stem cell transplantation [38]. It is possible that CsA and tacrolimus cause nephrotoxicity and hypomagnesemia through 2 totally unrelated mechanisms such that tacrolimus causes less nephrotoxicity thereby unlinking the association between hypomagnesemia and nephrotoxicity in tacrolimus-treated patients.

Our findings are surprising given the vast literature linking hypomagnesemia with negative outcomes. It might seem counterintuitive that declining renal function is associated with progressive endothelial dysfunction, as it is likewise associated with increasing serum Mg levels. This paradox is, however, explained by the presence of toxins accumulating in CKD with a much stronger effect on the endothelium such as asymmetric dimethyl arginine [39, 40], which predicts cardiovascular events in CKD/dialysis patients. Also, despite an increase in total serum Mg with decreasing kidney function, the biologically active ionized fraction is often lower in dialysis patients than in the general population, partially through complexing of Mg with phosphate and other anions [41]. In addition, serum Mg only constitutes a small fraction of total body Mg and poorly reflects the status of this largely intracellular cation in uremic patients [42]. An Mg deficit, irrespective of serum Mg levels, might exert negative effects on endothelial function and as such might contribute to renal injury and CVD.

Drawing conclusions from studies carried out in the general [43] CKD/ESRD populations [44–47] and extrapolating them to the transplant population may also be misleading. In fact, in kidney transplant recipients, only a small retrospective study limited to patients with biopsy-proven cyclosporine nephrotoxicity showed an association between hypomagnesemia and a faster decline in allograft function [48]. It is plausible that although hypomagnesemia has negative outcomes in CKD/ESRD patients, it reflects better tubular graft function early posttransplant and is thus associated with an improved allograft function in the long term. Our finding of low phosphate at 3 months and 1 year posttransplant in the low Mg group compared to the high Mg group, without any significant difference in PTH and calcium levels between the groups, supports this hypothesis. Hypomagnesemia as well as hypophosphatemia early posttransplant may be indicative of better tubular graft function. Beyond that, phosphate depletion can further enhance Mg loss by reducing Mg ion reabsorption in the distal convoluted tubule [49, 50].

Mg is important for immune responses and signaling events in immune cells such that in the absence of Mg, the function of immune cells is compromised. Free Mg has been implicated in T-cell activation [51]. Mg deficiency in the T-cell receptor signaling appears to lead to impaired phospholipase C γ 1 activation [52]. Despite these defects in T-cell activation, the B cells in these patients remained unaffected. On the other hand, Mg supplementation was able to decrease the secretion of type 2 CD4⁺ T-lymphocyte cytokines [53]. All of these suggest a crucial role of Mg in modulating the immune response, hinting to a possible role of hypomagnesemia in either controlling excessive T-cell-mediated immune responses in over-immunosuppressed transplant recipients or in impairing the immune response to prevent rejection.

Certain limitations should be considered when interpreting the results of this study. This study is an observational study performed retrospectively using the data from a large data registry. Observational analysis of a data registry shows the association (but not the causative relationships) between the primary variables of interest and outcome. Even though we adjusted for numerous variables, residual confounding parameters may still exist. The large number of patients involved, the long follow-up period, and standardization of treatment practices limit confounding. This is a retrospective study, which often deliberates a choice of immunosuppressive agents, possibly leading to inclusion bias. It does not, however, abolish the relationship between hypomagnesemia and graft function, nor that between the use of CNIs and hypomagnesemia. It might be claimed that the measurement of ionized and/or intracellular Mg levels may be a more reliable indicator of body Mg stored than the total Mg

measured in this study. Due to our study design, we did not perform ^{31}P nuclear magnetic resonance spectroscopy, the gold standard technique for measurement of free Mg. However, previous data pointed to a correlation between total serum Mg levels and intracellular free Mg levels measured by nuclear magnetic resonance spectroscopy in hypomagnesemic subjects ($r = 0.54$; $p < 0.001$) [54]. Also, it was shown that in renal transplant recipients, Mg levels were lower than those of control subjects whether they were measured as plasma (total and ionized) levels or intracellular erythrocyte and platelet Mg concentrations [55].

This study is the first to show that hypomagnesemia from 1 to 12 months after renal transplantation is associated with a better allograft function up to 5 years posttransplant in a large cohort of renal transplant recipients. This relationship remained significant even after adjustment for relevant clinical factors including use of loop diuretics; medical history of HTN, HF, and CVD; rejection rate, baseline allograft function; and the presence of SGF. A strong relationship between the use of CNIs and hypomagnesemia was demonstrated, which can potentially explain the discrepancy seen between univariate and multivariate analyses regarding the use of CNIs to prevent rejection and preserve graft function. The effects of CNIs on graft function may be partially attributed to CNI-induced hypomagnesemia. We believe that hypomagnesemia could serve as a biomarker of a better tubular function early on and predict a good long-term graft function. Future research should further elucidate the mechanisms by which hypomagnesemia exerts its protective effects on the allograft.

Statement of Ethics

This study was approved by the local Ethics Committee at BWH (protocol #: 2013P002558).

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Tammy Hod: planning, data analysis, and writing; Ofer Isakov: data analysis; Bhanu K. Patibandla: data organization and analysis; Kenneth B. Christopher: review; Rami Hershkovitz: review; Idit F. Shwartz: review; Anil Chandraker: planning and review.

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