

THE EFFECTS OF CNI AND MTORI-BASED REGIMENS ON BONE MINERAL DENSITY AFTER RENAL TRANSPLANTATION

Berfu Korucu, Hasan Yeter, Galip Guz

Gazi University Faculty of Medicine, Department of Nephrology, Ankara, Turkey

Corresponding author: Berfu Korucu, MD, Mailing address: Gazi Universitesi Tıp Fakültesi Hastanesi, Nefroloji BD, C Blok 2. Kat, Bahcelievler, Ankara, Turkey, Mobile phone: +905315262449, E-mail: berfukorucu@gmail.com

ABSTRACT

Background: Since glucocorticoids are used in low maintenance doses today, the relationship between calcineurin inhibitors (CNI) and osteoporosis has become clinically significant in osteoporosis after solid organ transplantation. However, there is evidence that the mammalian target of rapamycin inhibitors (mTORi) may be beneficial via osteoclast inhibition.

Objective: The bone mineral density (BMD) changes are investigated in renal transplant patients under CNI or mTORi-based maintenance regimens during the first five-year post-transplant course.

Methods: This study consists of thirty-three renal allograft recipients with less than one year of dialysis history. The exclusion criteria were: being older than 50 years old, history of bisphosphonate use, parathyroidectomy, CNI-mTORi switch after the post-transplant third month, diuretic use, and history of malignancy. First and fifth-year BMD scores and simultaneous laboratory parameters were evaluated.

Results: CNI (n=21) and mTORi group (n=12) had similar demographics, dialysis vintages, first and fifth-year serum parathormone, calcium, phosphate, magnesium, alkaline phosphatase, and 25-OH-vitamin D levels. The femur neck scores of the CNI group decreased from -0.82 (± 0.96) to -1.52 (± 0.92) ($p=0.020$). We observed a significant decrease in the CNI group compared to the mTORi group [-0.70 (± 0.68) and 0.30 (± 0.36), respectively; $p<0.01$] when the BMD score changes were evaluated among years. The mean femur neck score of the mTORi group increased insignificantly from -1.13 (± 0.65) to -0.82 (± 0.56) at the fifth-year DXA scan ($p=0.230$). Similar trends were also observed in L1-4 scores.

Conclusion: Our study suggests that CNI-based treatment is associated with decreased femur neck BMD scores, and mTORi-based treatment tends to be beneficial in the post-transplant five-year follow-up.

Keywords: calcineurin inhibitors; mammalian target of rapamycin inhibitors; bone mineral density; renal transplantation

INTRODUCTION

Osteopenia and osteoporosis are highly prevalent complications after solid organ transplantation (SOT) due to the immunosuppressive (IS) agents used [1]. Renal allograft recipients are the most susceptible group to bone diseases,

as they carry high or low bone turnover disease inherited from the chronic kidney disease course [2]. In addition, up to 15% of renal transplantation patients are reported to develop osteoporosis in the post-transplant first year [3, 4].

Among the IS medications used after SOT, glucocorticoids are the best-known agents which adversely affect bone microarchitecture [5, 6]. However, we now have more alternatives to effective IS drugs such as calcineurin inhibitors (CNI) and mammalian target of rapamycin inhibitors (mTORi) for the management of SOT. Glucocorticoids are currently used in maintenance therapy at very low doses [7, 8]. CNIs also negatively affect bone health. CNIs are shown to increase the parathormone levels, reduce magnesium levels, and increase osteoclast and osteoblast activity. Such effects cause a net bone mass reduction [6, 9]. In contrast, mTORi agents have been shown to have an antiresorptive effect on bones by reducing osteoclastic activity in vitro [10-13]. Limited studies on renal transplantation patients also reported that using sirolimus is associated with lower bone resorption markers compared to cyclosporine A (CsA) and tacrolimus [10, 14]. No studies yet compare these agents' long-term effects on bone health.

This study investigated the bone mineral density (BMD) changes in a selected renal transplant patient group under CNI or mTORi-based maintenance treatment regimens during the first five-year post-transplant course.

MATERIAL AND METHODS

Ethics approval

The Research Institute's Committee on Human Research approved the study protocol.

Study design and participants

This retrospective observational study consists of renal allograft recipients with less than one year of dialysis history and at least five years of follow-up after renal transplantation at our institute. Patients older than 50 years old, those receiving CNI and mTORi combination regimens, patients with estimated glomerular filtration rates (eGFR) (calculated by the Chronic Kidney Disease Epidemiology Collaboration formula less than 30 ml/min/1.73m²), history of bisphosphonate treatment and parathyroidectomy were excluded. Women undergoing menopause were also excluded. Other exclusion criteria were: CNI to mTORi switch or vice versa after the post-transplant third month, hypo- or hyperthyroidism, diuretic use, and history of malignancy.

In our clinic, serum drug levels are closely followed, and necessary interventions are actively carried out. Higher levels are targeted in the first three months of post-transplantation: after the third month, the tacrolimus level is maintained at 3-7 ng/ml, cyclosporine 12-hour trough (C0) level at 50-150 ng/ml, everolimus level at 3-8 ng/ml, and sirolimus level at 8-12 ng/ml.

Patients' demographic features, primary renal disease, and dialysis vintage were evaluated. In addition, the lifetime cumulative glucocorticoid dose was calculated for each subject. This was calculated simultaneously with the first-year and fifth-year dual-energy X-ray absorptiometry (DXA) scan time, including the pre-transplantation period.

Materials and Measurements

Patients' laboratory parameters of the pre-transplantation period, first-year, and fifth-year post-transplantation simultaneous with the DXA scan time were evaluated retrospectively.

DXA scan results were scored with the unit of g/cm² to examine BMD of the posteroanterior position of left proximal femoral neck and lumbar vertebrae L1-4 (DXA Horizon; Hologic Inc., Marlborough, Massachusetts, USA). Since postmenopausal women and patients over 50 years were excluded, Z scores were evaluated in the analyses.

Statistical methods

The study's numeric values were shown with mean and standard deviation (SD), or medians with ranges, categorical data, frequency, and percentage. For data normally disturbed, the Unpaired Student's T-Test was used to compare two groups. The Kruskal-Wallis test was performed for abnormally distributed data. A p-value of less than 0.05 was considered statistically significant. The statistical analyses were performed using the Statistical Package for Social Science (SPSS, Chicago, IL, USA) for personal computers, version 21.0.

RESULTS

Patient characteristics

The CNI and mTORi groups had similar demographics and dialysis vintages (Table 1). No diabetic patients existed in either group, and the lipid profiles were similar. The cumulative

Table 1. Baseline characteristics of the patients with CNI and mTORi-based regimens

	CNI (n=21)	mTORi (n=12)	p-value
Age mean ($\pm$ SD)	38.1 ($\pm$ 6.3)	36.3 ($\pm$ 6.8)	0.443
Sex F/M (n)	5/16	4/8	0.569
BMI mean, kg/m ² (SD)	22.9 ($\pm$ 3.3)	24.1 ($\pm$ 4.0)	0.368
Kidney Disease (n)	Unknown: 8 Gn/Vasc: 4 DM: 0 Others: 9	Unknown: 4 Gn/Vasc: 2 DM: 0 Others: 6	0.725
Dialysis time (mo) median (min-max)	0 (0-11)	0 (0-10)	0.319
Albumin mean, g/dl ($\pm$ SD)	4.4 ($\pm$ 0.4)	4.5 ($\pm$ 0.6)	0.375
LDL mean, mmol/l ($\pm$ SD)	2.74 ($\pm$ 0.78)	3.86 ($\pm$ 1.08)	0.539
Triglyceride mean, mmol/l ($\pm$ SD)	1.59 ($\pm$ 0.36)	1.68 ($\pm$ 0.81)	0.622
Cum Gc 1 st year mean, mg ($\pm$ SD)	3422.3 ($\pm$ 442.6)	3199.3 ($\pm$ 810.7)	0.313
Cum Gc 5 th year mean, mg ($\pm$ SD)	3645.2 ($\pm$ 478.6)	3379.5 ($\pm$ 863.9)	0.262
Pre Tx iPTH mean, ng/l ($\pm$ SD)	449.3 ($\pm$ 246.9)	452.9 ($\pm$ 202.1)	0.966
Donor type (n)	Deceased: 0 Living: 21 • Parent: 10 • Sibling: 6 • Spouse: 5 • Unrelated: 0	Deceased: 0 Living: 12 • Parent: 5 • Sibling: 3 • Spouse: 4 • Unrelated: 0	0.621
Medication (n)	Tac: 17 CsA: 4	Eve: 8 Siro: 4	-

CNI: calcineurin inhibitor, mTORi: mammalian target of rapamycin inhibitor, BMI: body mass index, Gn/Vasc: Glomerulonephritis or systemic vasculitis, DM: Diabetes mellitus, mo: months, LDL: low-density lipoprotein, Cum Gc: cumulative glucocorticoid dose, Pre Tx iPTH: pre-transplantation intact parathormone, Tac: tacrolimus, CsA: cyclosporine A, Eve: everolimus, Siro: sirolimus

glucocorticoid doses were calculated until the patients' post-transplant first and fifth-year DXA scans. Doses were similar between the groups. The groups each had similar pre-transplantation median iPTH levels. All patients had living donors. The donor type distribution was also similar between groups, as shown in Table 1.

Bone health-related laboratory parameters of the patients

The bone health-related laboratory values of the patients are simultaneous to the dates of the post-transplant first and fifth-year DXA scan. Results are shown in Table 2. The groups had similar mean eGFR values, mean iPTH, ALP, total calcium, serum phosphorus, magnesium levels. The

groups had similar mean 25-OH-Vitamin D levels at the post-transplant first year and fifth year.

Bone mineral density scores of the groups

Table 3 and Figure 1 show the BMD scores and comparisons within and between the groups. The mean femur neck score of the CNI group significantly decreased from -0.82 ($\pm$ 0.96) to -1.52 ($\pm$ 0.92) at the fifth-year DXA scan ($p=0.020$). An insignificant decrease existed from -0.78 ($\pm$ 1.57) to -0.99 ($\pm$ 1.33) in the mean lumbar L1-L4 score of the CNI group ($p=0.643$).

The mean femur neck score of the mTORi group insignificantly increased from -1.13 ($\pm$ 0.65) to -0.82 ($\pm$ 0.56) at the fifth-year DXA scan ($p=0.230$). The mean lumbar L1-L4 score of

Table 2. Bone health-related laboratory parameters of the patients

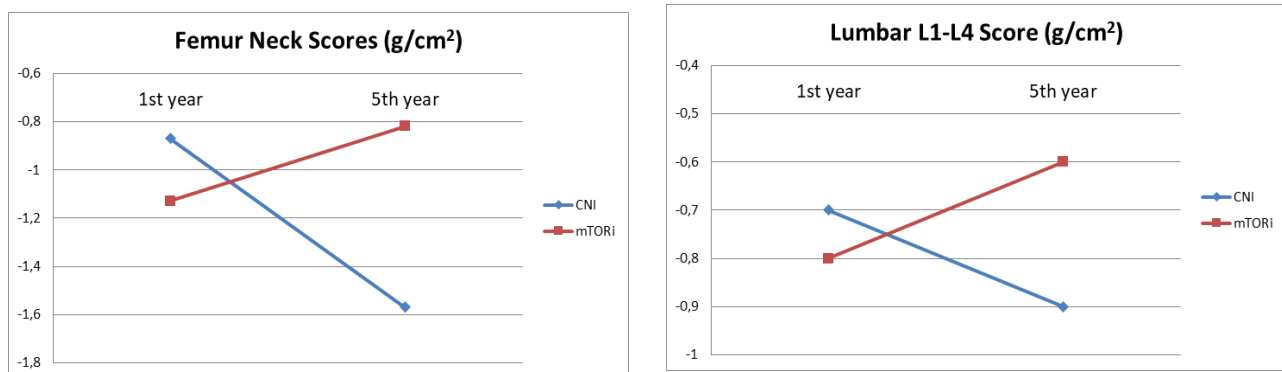
	Post-transplant 1 st year			Post-transplant 5 th year		
	CNI	mTORi	p-value	CNI	mTORi	p-value
eGFR mean, ml/min/1.73m ² (SD)	72.1 (±23.0)	72.3 (±18.6)	0.982	74.8 (±23.4)	70.7 (±15.4)	0.592
HCO ₃ ⁺ mean, mmol/l (SD)	23.2 (±2.4)	22.9 (±1.9)	0.644	22.4 (±2.1)	22.4 (±2.3)	0.989
iPTH mean, ng/l (SD)	66.4 (±29.4)	63.7 (±26.4)	0.794	62.6 (±25.0)	67.0 (±29.9)	0.658
ALP mean, µkat/l (SD)	1.40 (±0.64)	1.32 (±0.50)	0.705	1.37 (±0.58)	1.27 (±0.43)	0.598
Ca mean, mmol/l (SD)	2.40 (±0.12)	2.35 (±0.13)	0.416	2.38 (±0.14)	2.36 (±0.14)	0.930
P mean, mmol/l (SD)	1.04 (±0.20)	1.20 (±0.48)	0.143	1.06 (±0.13)	1.01 (±0.19)	0.335
Mg mean, mmol/l (SD)	0.65 (±0.07)	0.66 (±0.12)	0.464	0.72 (±0.08)	0.74 (±0.08)	0.305
25-OH-VitD mean, nmol/l (SD)	66.6 (±26.2)	63.9 (±20.7)	0.740	67.4 (±16.7)	62.4 (±16.4)	0.730

CNI: calcineurin inhibitors, mTORi: mammalian target of rapamycin inhibitors, eGFR: estimated glomerular filtration rate, HCO₃⁺: serum bicarbonate, iPTH: intact parathormone, ALP: alkaline phosphatase, Ca: total calcium, P: phosphate, Mg: magnesium, 25-OH-VitD: 25 hydroxyvitamin D

Table 3. Bone mineral densities of patients and comparison of changes in the scores over years

		Post-transplant 1 st year	Post-transplant 5 th year	p-value	Δ BMD score	p-value
Femur Neck mean, g/cm ² (SD)	CNI	-0.82 (±0.96)	-1.52 (±0.92)	0.020	-0.70 (±0.68)	<0.001
	mTORi	-1.13 (±0.65)	-0.82 (±0.56)	0.230	0.30 (±0.36)	
Lumbar L1-L4 mean, g/cm ² (SD)	CNI	-0.78 (±1.57)	-0.99 (±1.33)	0.643	-0.20 (±1.45)	0.269
	mTORi	-0.89 (±1.15)	-0.60 (±1.09)	0.532	0.29 (±0.64)	

CNI: calcineurin inhibitors, mTORi: mammalian target of rapamycin inhibitors, BMD: bone mineral density



CNI: calcineurin inhibitors, mTORi: mammalian target of rapamycin inhibitors

CNI: calcineurin inhibitors, mTORi: mammalian target of rapamycin inhibitors

Figure 1. Changes in the post-transplantation bone mineral density scores

the mTORi group also insignificantly increased from $-0.89 (\pm 1.15)$ to $-0.60 (\pm 1.09)$ ($p=0.532$).

We observed a significant decrease in the CNI group compared to the mTORi group [$-0.70 (\pm 0.68)$ and $0.30 (\pm 0.36)$, respectively; $p < 0.01$] when the BMD score differences (Δ BMD score) of the femoral neck and lumbar L1-L4 values between the first and fifth years of the post-transplant patients were evaluated. No difference was observed in the lumbar L1-L4 score differences (Δ BMD score) [$-0.20 (\pm 1.45)$ and $0.29 (\pm 0.64)$, respectively; $p=0.269$].

DISCUSSION

Our study results suggest a significant decrease in femur neck BMD scores during the post-transplant five-year in patients receiving a CNI-based treatment compared to mTORi-based treatment. The BMD scores did not decrease, and even a slight improvement was observed in the patients receiving an mTORi-based treatment. When the first-year and fifth-year BMD score changes were calculated, a significant difference was discovered between groups favoring the mTORi group.

The animal models reveal that CsA increases bone formation and resorption rates, causing a high-turnover bone disease. Tacrolimus also causes bone loss without changing the turnover rate [15]. Despite the existence of controversy, the relationship between the CNIs and osteoporosis was reported in human studies [16-19]. In contrast, preclinical studies demonstrated a bone protective effect of mTOR pathway inhibition. mTORi inhibits bone resorption, increases osteoclast apoptosis, and promotes in vitro osteoblast survival rate [11-13, 20].

Campistol et al.'s study is the first to compare the effects of CNI and mTORi in renal allograft recipients as the secondary end-point of two phase-2 trials comparing CsA and sirolimus. After a one-year follow-up, they found that the urinary N-telopeptides and serum osteocalcin were consistently and significantly higher in the CsA treatment group than the sirolimus treatment group [14]. As suggested in the preclinical studies, the authors concluded that sirolimus might be related to less bone turnover and resorption. Secondly, the cross-sectional study of Westfeld et al. revealed significantly higher bone re-

sorption markers osteoclast-specific TRAP isoform 5b and soluble receptor activator of nuclear factor kappa-B ligand (sRANKL), and similar osteoprotegerin levels existed in the CNI-based treatment group when compared to the sirolimus-based treatment group [10]. They also investigated osteoclast precursor proliferation in a cell culture sample treated with tacrolimus, sirolimus, and mycophenolate mofetil (MMF). Sirolimus reduced the number of osteoclasts, but tacrolimus and MMF had no effect. Although few clinical studies support the preclinical findings, to date, there is no study evaluating the long-term effects of CNI and mTORi on bone health in renal transplantation patients.

Our study's broad exclusion criteria facilitated the study groups' comparison of bone disease, a highly complex subject among this population. For example, patients over 50 years old or who have undergone renal transplantation after several years of dialysis treatment make it difficult to distinguish between the impact of the IS medications' side effects and the age or illness-induced effects. However, the width of our exclusion criteria resulted in a relatively small sample size. We may have underestimated the findings in the mTORi group, which did not provide statistical significance, but they did show slight BMD score improvements over time. This finding is consistent with those in the in vitro and animal models. Further studies with broad samples may support this positive trend on the BMD scores of mTORi-based treatment we observed in the long-term follow-up.

Other limitations of our study are its retrospective design and the use of BMD data as the indicator of bone health. Although its practical use is quite limited, it is clear that bone biopsy is the best indicator of bone health in individuals with a history of chronic kidney disease. However, since our study is the first in the literature to test a hypothesis based on the data of previous preclinical studies, we have obtained data that will inspire prospective studies with bone biopsy and other bone resorption markers.

According to the results of our study, CNI-based treatment is associated with decreased femur neck BMD scores, and mTORi-based treatment tends to be beneficial in terms of mineral bone disease in the five-year follow-up of renal transplantation patients. Therefore, more studies with broad samples are necessary to confirm our results.

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Резиме

ЕФЕКТИТЕ НА РЕЖИМИТЕ ЗАСНОВАНИ НА CNI И mTORi ВРЗ МИНЕРАЛНАТА ГУСТИНА НА КОСКИТЕ ПО БУБРЕЖНА ТРАНСПЛАНТАЦИЈА

Берфу Коруцу, Хасан Јетер, Галип Гуз

Медицински факултет на Универзитетот Гази, Катедра за нефрологија, Анкара, Турција

Историјат: Бидејќи глукокортикоидите денес се користат во ниски дози на одржување, врската меѓу инхибиторите на калцинеурин (CNI) и остеопорозата стана клинички значајна кај остеопорозата по трансплантација на цврсти органи. Сепак, постојат докази дека целта на инхибиторите на рапамицин кај цицачите (mTORi) може да биде корисна преку инхибиција на остеокластите.

Цел: Промените во коскената минерална густина (BMD) се испитуваат кај пациенти со бубрежна трансплантација под режими на одржување базирани на CNI или mTORi во текот на првите пет години по трансплантацијата.

Методи: Оваа студија се состои од триесет и тројца приматели на бубрежен алографт со помалку од една година историја на дијализа. Критериумите на исклучување беа: да се биде постар од 50 години, историја на употреба на бифосфонати, паратиroidектомија, CNI-mTORi префрлување по третиот месец од трансплантацијата, употреба на диуретици и историја на малигнитет. Беа евалуирани резултатите на BMD од првата и од петтата година и истовремените лабораториски параметри.

Резултати: CNI (n = 21) и mTORi групите (n = 12) имаа слична демографија, нивоа на дијализа, серумски паратормон од првата и од петта година, калциум, фосфат, магнезиум, алкална фосфатаза и нивоа на 25-ОН-витамин Д. Резултатите на вратот на бедрената коска на групата CNI се намалија од $-0,82 (\pm 0,96)$ на $-1,52 (\pm 0,92)$ ($p = 0,020$). Забележавме значително намалување во групата CNI во споредба со групата mTORi [$-0,70 (\pm 0,68)$ и $0,30 (\pm 0,36)$, соодветно; $p < 0,01$], кога промените во резултатот на BMD беа евалуирани низ годините. Просечниот резултат на вратот на бедрената коска на групата mTORi незначително се зголеми од $-1,13 (\pm 0,65)$ на $-0,82 (\pm 0,56)$ на петгодишното DXA скенирање ($p = 0,230$). Слични трендови беа забележани и во оцените L1-4.

Заклучок: Нашата студија сугерира дека третманот заснован на CNI е поврзан со намалени резултати на BMD на вратот на бедрената коска, а третманот базиран на mTORi има тенденција да биде корисен во петгодишното следење по трансплантацијата.

Клучни зборови: инхибитори на калцинеурин, цел на инхибиторите на рапамицин кај цицачите, минерална густина на коските, бубрежна трансплантација