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Original Article

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Sarcopenia Susceptibility in Kidney Transplant Recipients: A Cross-Sectional Study Investigating the Impact of the Mammalian Target of Rapamycin Inhibitors

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ABSTRACT

Background: Dysfunction of the mammalian target of the rapamycin (mTOR) signaling pathway by mTOR inhibitors in kidney transplant recipients (KTRs) may contribute to sarcopenia in addition to metabolic factors and inflammation. Our study aimed to investigate the association of mTOR inhibitors with sarcopenia ın KTRs treated with and without mTOR inhibitors.

Methods: The study included 22 KTRs who had been on mTOR inhibitors for at least 6 months and 51 KTRs who had never been on mTOR inhibitors. Handgrip strength (HGS) was used to test muscle strength. Bioimpedance analysis (BIA) and muscle ultrasonography (US) were used to determine muscle mass.

Results: The study population's mean age was 39.05 ± 13.29 years, and 41.1% of the participants were female. One out of every 3 patients was under treatment with mTOR inhibitors, either everolimus or sirolimus. Sarcopenia was found in 32.9% of the whole population, according to EWGSOP2 criteria. There was no difference in the prevalence of sarcopenia ($P = .68$) and its components ($P > .05$) between the mTOR-inhibitor-using and mTOR-inhibitor-free groups. In regression analysis, mTOR inhibitors were not associated with sarcopenia ($P = .68$).

Conclusion: Muscular strength, mass, and physical performance did not differ between the mTOR-inhibitor-using and mTOR-inhibitor-free groups, implying that changes in molecular pathways may not necessarily translate into clinical manifestations in real life.

Keywords: Kidney transplantation, sarcopenia, mTOR inhibitors

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INTRODUCTION

Sarcopenia is a progressive and systemic condition of the skeletal muscles that causes a loss of muscular strength and mass. Sarcopenia is significantly linked to a number of negative outcomes, including falls, fractures, physical impairment, and death. Sarcopenia is a disorder typically associated with older ages; however, it can also arise at younger ages due to chronic conditions[1,2] and one of them is chronic kidney disease. According to the National Health and Nutrition Examination Survey (NHANES) III research, the prevalence of sarcopenia increases as the glomerular filtration rate declines.[3] Recent studies indicate that kidney transplantation recipients (KTRs) also suffer from sarcopenia.[4,5] In kidney transplantation, sarcopenia increases morbidity, mortality, and the incidence of cardiovascular problems.[6] It has also been demonstrated that decreased muscle mass is connected with a poor quality of life in KTRs.[7]

Various studies have found that the prevalence of sarcopenia in KTRs ranges from 11.1% to 49.6%.[4,5,8,9] This variability in sarcopenia prevalence may be related to differences in KTRs, such as age, sex, race, and

comorbidities, or to the use of diverse criteria for sarcopenia. Muscle mass declined at 1 month post-transplantation and returned to pre-transplantation values over the course of 1 year; age and body mass index (BMI) may be the risk factors for this change in muscle mass among these patients.[10] Pretransplant dialysis, hormonal and immune system alterations, muscle inflammation, metabolic acidosis, decreased protein consumption, lack of physical activity, elevated levels of angiotensin II, abnormalities in insulin/insulin-like growth factor I, and myostatin expression, collectively play a role in the onset of muscle wasting.[11] A decrease in the function of satellite cells raises the likelihood of sarcopenia in KTRs. In addition, the administration of immunosuppressive medications, primarily systemic corticosteroids, induces sarcopenia.[12,13]

Mammalian target of rapamycin (mTOR) inhibitors are an additional class of immunosuppressive medications used for kidney transplantation. Skeletal muscle protein synthesis is regulated by the mTOR kinase and its downstream effectors, Akt/Protein Kinase B (PKB). There are three homologous isoforms of the serine/threonine kinase Akt: Akt1, Akt2, and Akt3. In skeletal muscle, Akt1 and Akt2 are expressed to a greater degree than Akt3.[14] The significance of Akt/PKB in skeletal muscle was demonstrated by measuring fiber hypertrophy and enhancing denervation-induced atrophy in response to its in vivo overexpression.[15] mTORC1 and mTORC2 are the 2 types of mTOR complexes present in a cell. mTORC1 regulates cell proliferation, protein synthesis, and autophagy via the phosphorylation of downstream effectors, such as the translation regulators S6K1 and 4E-BP1.[16] The discovery of the effect of mTOR complexes on protein synthesis via multiple pathways has raised interest in the possible clinical effects of mTOR inhibitors on muscle mass in real life.

Consequently, the effect of mTOR inhibitors on muscle protein synthesis and its clinical manifestation is controversial. Even though mTOR inhibitors are often prescribed in kidney transplantation, the effects of mTOR inhibitors on muscle in this particular patient population have not been thoroughly studied. This proposed study aims to elucidate the potential relationship between mTOR inhibitors and sarcopenia in KTRs.

MAIN POINTS

- Many different factors cause muscle wasting in kidney transplantation patients. Age, phase angle measured by a bioimpedance analyzer (BIA), and body mass index are independently associated with sarcopenia in kidney transplantation recipients.
- Muscle ultrasonography can be used to identify sarcopenia in kidney transplantation recipients.
- Evaluation of muscle mass by both BIA and ultrasonography has revealed that mammalian target of rapamycin (mTOR) inhibitors had no clinical impact on muscle mass in kidney transplantation recipients.

MATERIAL AND METHODS

Study Population

This cross-sectional study was conducted on 73 KTRs with deceased or living-related donors who consented to participate and were currently receiving treatment at our hospital's outpatient kidney transplantation clinic between December 2020 and December 2021. Our study included 22 KTRs who were treated with mTOR inhibitors for at least six months and 51 KTRs who received various immunosuppressive therapies (mycophenolate mofetil, azathioprine, tacrolimus, cyclosporine, and prednisolone) but never used mTOR inhibitors. Indications for mTOR inhibitors for the study population appeared to be cases requiring the replacement of antiproliferative agents, such as BK virus nephropathy, or requiring the replacement of calcineurin inhibitors (CNI), such as CNI toxicity or selected cases of chronic allograft nephropathy. Participants' demographic information was obtained. All participants' primary kidney disease, kidney transplantation date, donor type, kidney replacement therapy history (hemodialysis/peritoneal dialysis), rejection history, immunosuppressive drugs, and medication history were documented. Using a wall-secured stadiometer, a digital scale, and a retractable measuring tape, height, weight, calf, mid-arm, waist, and hip circumference measurements were respectively taken, and BMI was determined. All patients' 24-hour protein excretion on the day of the evaluation was recorded, and 0.15 g/day urine protein excretion was defined as proteinuria. To evaluate the graft function, serum albumin, serum creatinine levels, and glomerular filtration rates (upper limit of 60 mL/min/1.73m^2) were noted at the time of measurement.

Exclusion Criteria

Patients who had received high-dose corticosteroid treatment in the previous month or were on any mTOR inhibitor therapy for less than 6 months were not eligible for inclusion in the study. Patients with malignancy, autoimmune disease, acute decompensating condition, amputation, liver failure, or mental illness; pregnant or nursing women; KTRs on dialysis; and those unable to complete a performance test were also excluded. Bioelectrical impedance analysis (BIA) was not performed on patients with apparent edema, intracardiac defibrillator, or any other implantation.

Sarcopenia Assessments

Muscle strength

Handgrip strength (HGS) was evaluated from the dominant/non-fistula arm while the patients stood with their arms at a position parallel to the floor using a calibrated handheld dynamometer (T.K.K.5401; Takei Scientific Instruments, Tokyo, Japan). Handgrip strength was measured three times at 1-minute intervals on both hands. In the end, the best performance out of the three measurements was selected for the statistical analysis. Low HGS (probable sarcopenia) was defined as 23 kg or less for females and 39 kg or less for males.[17]

Performance Tests

Low physical performance was determined by a gait speed that was less than 0.8 m/sec when measured with a manual stopwatch over a distance of 4 meters. For determination the patient was instructed to walk in the indicated area at a rate that was comfortable for them. The calculation for gait speed was based on the average of two separate assessments.

Muscle Mass Evaluations

Bioimpedance Analysis

All individuals underwent an assessment of their body composition using the BodyStat Quadscan 4000 BIA, a device designed to measure various aspects of body composition. The measurements were conducted with a multifrequency and tetrapolar technique, and participants were in a supine position after fasting overnight. The analyzer directly yielded data on fat-free mass (FFM). Additionally, appendicular skeletal muscle mass (ASMM) was calculated using reactance and resistance values obtained through BIA, employing the equation formulated by Kyle et al. The ASMM plays a fundamental role in evaluating muscle mass and potential muscle depletion.

To identify sarcopenia, the study employed the appendicular skeletal muscle mass index (ASMI), calculated by dividing ASMM in kilograms by the square of the participant's height in meters. This index helps in determining the presence of sarcopenia, a condition characterized by muscle loss. The study also documented the BIA-derived phase angle (PA) for all participants. The PA is a parameter that can offer insights into cell membrane integrity and overall health status.

Ultrasonographic Measurements

Muscle ultrasonography represents a robust and scientifically sound method for quantifying muscle mass, in accordance with the guidelines provided by the Sarcopenia Special Interest Group of the European Union Geriatric Medicine Society.[18] The ultrasonographic assessment encompassed the examination of five specific muscle groups, the medial head of gastrocnemius, rectus femoris (RF), rectus abdominis (RA), external oblique (EO), internal oblique (IO), and transversus abdominis (TA), employing an 8-10 MHz linear probe with a 5 cm width (LOGIQ 200 PRO, General Electric Medical Systems).

All measurements were conducted by a single, seasoned physician who remained unaware of the study's outcomes. Furthermore, each measurement was taken on the right side of the body, with the ultrasound probe being applied with the least possible pressure. To account for respiratory effects, imagery of the trunk muscles (RA, EO, IO, and TA) was acquired upon the completion of a standard exhalation. In the case of RF muscle thickness, the widest gap between the superficial and deep fascia was recorded in a transverse image. Whenever possible, the cross-sectional area (CSA) of the muscle was measured, which is defined as the area of the cross-section perpendicular to its long axis.

Sarcopenia Diagnosis

The diagnosis of sarcopenia adhered to the EWGSOP2 criteria, which considers low muscle mass, diminished muscle strength, and reduced physical performance, as indicated by the specified threshold values provided above. The presence of low HGS is indicative of probable sarcopenia, while sarcopenia is defined by the EWGSOP2 criteria as the combination of low muscle strength and low muscle mass.[19]

Statistical Analysis

The statistical analysis was conducted using Statistical Package for Social Science Statistics, version 26.0 (IBM SPSS Corp.; Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages. The distribution of characteristics was determined using the Kolmogorov–Smirnov test, and the homogeneity of variances was assessed with Levene's test. Continuous variables were presented as either means ± standard deviation (SD) or as a median and interquartile range, depending on the normality of their distribution. To compare the data, the chi-square test or Fisher's exact test was employed for categorical variables, while the Mann–Whitney *U*-test or Student's *t*-test was used for continuous variables. Correlation analyses, where applicable, utilized Pearson and Spearman's coefficients. To identify independent predictors of sarcopenia, a binary logistic regression model employing the backward method was employed. Model 1, an unadjusted model, solely considered the use of mTOR inhibitors. Model 2 included age, sex, and mTOR inhibitor treatment as components. In model 3, kidney transplantation-related factors were also taken into consideration. The model fit was evaluated using appropriate residual and goodness-of-fit statistics. A *P*-value of .05 was established as the threshold for statistical significance.

Ethical Disclosures

Ethical Committee of our Hacettepe University Faculty of Medicine granted approval for the study (with the decision number: 2021/13-01). Written informed consent was obtained from all participants or their legal guardians, following the provision of written information about the study. The study protocol was aligned with the principles established in the Declaration of Helsinki.

RESULTS

The mean age of the study population was 39.05 ± 13.29 years, and 41.1% of the participants were female. Only 8 patients had a functioning arteriovenous fistula on nondominant hands, and none of the participants had a functioning AVF on dominant hands. One of every three patients was under treatment with mTOR inhibitors (30.1%, n = 22), either everolimus or sirolimus. On the other hand, 69.9% of patients (n = 51) were using other immunosuppressive therapies. Sarcopenia was found in 32.9% of the whole population according to EWSGOP2 criteria. According to their mTOR inhibitor therapy status (mTOR

inhibitor-using group and mTOR inhibitor-free group), the two groups were similar by age and sex distribution (*P* = .44 and *P* = .44, respectively). In addition, there was no statistically significant difference between the groups regarding BMI (*P* = .46). There were no differences between the two groups with regard to primary kidney disease, rejection episodes, duration of transplantation, type of donor, kidney replacement therapy before transplantation, or proteinuria (*P* > .05 for all). The most commonly used immunosuppressive medications in mTOR inhibitor-free group were tacrolimus (78.4%) and mycophenolate mofetil (60.8%). There were no differences in terms of graft functions between these two groups. The median duration of use of everolimus was 19.0 [8.0-70.0] and for sirolimus it was 74.5 [71.0-82.5] months. The average 6-month serum levels of everolimus and sirolimus were found to be 4.56 [4.09-4.90] and 10.05 [6.65-12.80], respectively. The demographic and characteristic factors of the two groups are shown in Table 1.

The muscle assessments are presented in Table 2. No differences between the two groups were found when comparing anthropometric measurements, HGS, or sarcopenia measured by muscle US or BIA. Sarcopenia was found in 36.4% of patients (n = 8) in the mTOR-using group and 31.4% (n = 16) of patients in the mTOR inhibitor-free group were sarcopenic (*P* = .78).

The entire study population was separated into sarcopenic and nonsarcopenic groups. In the sarcopenic group, 50.0% of the patients were female, whereas in the nonsarcopenic group, the female ratio was 36.7% (n = 18). The sarcopenic group was older than the nonsarcopenic group, although the difference was not statistically significant (mean age 43.88 ± 15.45 years versus 36.69 ± 11.1 years; *P* = .051). There was no difference in BMI and unintentional weight loss. In terms of primary kidney disease, rejection episodes, time since transplant, type of donor, kidney replacement therapy before transplantation, and proteinuria, there were no statistically significant differences between the sarcopenic and nonsarcopenic groups (Table 3). The sarcopenic group had a substantially smaller calf circumference than the nonsarcopenic group (*P* = .003). Using muscle US, the thickness of sarcopenic patients' upper thigh and RA muscles was significantly smaller than that of nonsarcopenic individuals. Patients with sarcopenia had a decreased median PA, and the difference was statistically significant (*P* = .001).

Multivariable binary logistic regression analysis is shown in Table 4. The use of mTOR inhibitors was not statistically correlated with sarcopenia in the unadjusted model or after being adjusted for age and sex (*P* = .68, OR: 1.25 and 95% CI, 0.46-3.56). Age increased the incidence of sarcopenia significantly independent of kidney transplantation characteristics according to model 3 (*P* = .047, OR: 1.051 and 95% CI, 1.001-1.105).

Correlation analyses were performed to examine the relationship between handgrip strength, SMI measured in BIA, and

Table 1. Baseline Characteristics of the Study Population According to mTOR Treatment Status

	mTOR Inhibitor-Using Group (n = 22)	mTOR Inhibitor-Free Group (n = 51)	*P*
Sex, female	7 (31.8)	23 (45.1)	.29
Age, years	37.23±12.3	39.84±13.7	.44
BMI, kg/m^2	25.4±4.9	26.4±5.4	.46
Education, ≥8 years	12 (54.5)	29 (56.9)	.86
Smoking history	3 (13.6)	7 (14.3)	.38
Primary kidney disease			.40
Kidney agenesis	4 (18.2)	6 (11.8)	
Nephritic/nephrotic	3 (13.6)	17 (33.3)	
syndrome	2 (9.1)	4 (7.8)	
Diabetes mellitus	8 (36.4)	12 (23.5)	
Unknown	4 (18.2)	4 (7.8)	
Vesicourethral reflux	1 (4.5)	3 (5.9)	
Nephrolithiasis	–	7 (9.1)	
Other			
Graft rejection	7 (33.3)	10 (20.4)	.25
Dialysis prior to transplantation	14 (63.6)	35 (68.6)	.59
Type of donor			.25
Deceased donor	4 (18.2)	16 (31.4)	
Living-related donor	18 (81.8)	35 (68.6)	
Posttransplant time in months	102.0 [46.25-138.50]	87.0 [55.25-117.0]	.71
Immunosuppressive therapies	7 (31.8)	31 (60.8)	.082
Mycophenolate	-	13 (25.5)	-
mofetil	16 (72.7)	40 (78.4)	.50
Azathioprine	2 (9.1)	9 (18.3)	.50
Tacrolimus	22 (100.0)	50 (98.0)	1.0
Cyclosporine			
Prednisolone			
Serum Albumin, g/dL	4.25 [4.06-4.47]	4.38 [4.10-4.58]	.39
Creatinine, mg/dL	1.22 [1.14-1.90]	1.21 [1.05-1.53]	.31
Glomerular filtration rate ml/min/1.73 m^2	60 [34.4-60.0]	60 [50.4-60.0]	.42
Average blood everolimus level of 6 months (ng/ml)	4.56 [4.09-4.90]	–	
Duration of everolimus use, months	19.0 [8.0-70.0]		
Average blood sirolimus level of 6 months (ng/ml)	10.05 [6.65-12.80]		
Use of sirolimus duration, months	74.5 [71.0-82.5]		
Presence of Proteinuria	6 (27.2)	13 (25.5)	.94
Unintentional weight loss (kg)	5 (22.7)	12 (23.5)	.94

Variables are given as n (%), mean ± SD, or median [Q1-Q3].
BMI, body mass index.

Table 2. Muscle Assessments of Study Population According to Treatment Groups

	mTOR Inhibitor-Using Group (n = 22)	mTOR Inhibitor-Free Group (n = 51)	*P*
Calf circumference, cm	33.0 [29.7-34.2]	34.0 [31.0-37.0]	.29
Mid-arm circumference, cm	26.0 [23.8-30.0]	27.0 [25.0-31.5]	.30
Waist circumference, cm	88.5 [88.7-99.2]	88.0 [80.0-100.0]	.49
Hip circumference, cm	96.5 [91.5-106.0]	99.0 [89.0-108.0]	.66
HGS, kg	32.6 [25.6-38.6]	32.2 [21.7-41.5]	.82
Fat%	20.25 [15.9-27.6]	25.60 [17.4-32.6]	.14
FFMI, kg/m^2	19.05 [17.5-22.4]	18.50 [17.4-20.9]	.56
ASMI, kg/m^2	6.77 [5.4-7.8]	6.80 [5.5-8.3]	.67
Low muscle mass,	12 (54.5)	23 (45.1)	.46
Phase angle	6.25 [5.8-7.1]	6.50 [5.6-8.0]	.77
Gait speed, m/s	1.24 [0.92-1.45]	1.07 [0.90-1.35]	.19
Sarcopenia, %	8 (36.4)	16 (31.4)	.68
Rectus femoris muscle (milimeters)	17.1 [13.5-9.5]	16.3 [14.8-18.2]	.62
Rectus femoris CsA, cm^2	7.95 [5.8-8.6]	6.66 [5.9-9.1]	.79
Medial head of gastrocnemius muscle, mm	14.65 [13.0-17.1]	15.60 [13.9-17.8]	.18
Rectus abdominis muscle,mm	8.35 [6.7-9.5]	8.50 [6.7-9.5]	.86
External oblique muscle,mm	4.15 [3.6-4.7]	3.80 [3.1-4.5]	.14
Internal oblique muscle, mm	6.40 [4.6-8.2]	6.1 [5.0-7.5]	.56
Transversus abdominis muscle, mm	3.70 [3.1-4.2]	3.80 [3.4-4.5]	.27

Variables are given as n(%), mean ± SD, or median [Q1-Q3].
ASMI, appendicular skeletal muscle mass index; FFMI, fat-free mass index; mm, milimeter; cm^2, centimeter square; HSG, handgrip strength.

everolimus serum blood levels on the day of measurement. The results showed that everolimus levels were negatively and statistically correlated with HGS (rho: −0.515, *P*-value < .05). SMI was strongly and negatively correlated with everolimus levels (rho: −0.747, *P*-value < .05) (Figure 1).

DISCUSSION

Sarcopenia could be attributed to various factors, including the disruption of muscle protein synthesis. Our study revealed that there were no significant differences observed between the groups of individuals who were using mTOR inhibitors and those who were not, in terms of muscle mass, as assessed through both BIA and US, muscle strength, or physical performance. which was assessed by gait speed, a useful indicator that may be used to evaluate and monitor functional status and general health in a wide range of populations. Furthermore, our regression analysis did not find any correlation between the use of mTOR inhibitors and the presence of sarcopenia.

Table 3. Baseline Characteristics of the Study Population According to Presence of Sarcopenia

	Sarcopenic (n = 24)	Non-sarcopenic (n = 49)	*P*
Sex, female	12 (50.0)	18 (36.7)	.28
Age, years	43.88 ± 15.45	36.69 ± 11.55	.051
BMI, kg/m^2	24.75 ± 5.23	26.77 ± 5.12	.12
Education, ≥8 years	10 (41.7)	31 (63.3)	.081
Smoker	3 (13.6)	7 (14.3)	.70
Graft rejection	4 (17.4)	13 (27.7)	.35
Dialysis prior transplantation	16 (66.7)	33 (67.3)	.85
Type of donor			.81
Deceased	7 (29.2)	13 (26.5)	
Living	17 (70.8)	36 (73.5)	
Posttransplant time, months	74.0 [54.0-106.0]	59 [95.0-119.5]	.35
Proteinuria	3 (17.6)	10 (21.7)	.72
Unintentional weight loss	6 (25.0)	11 (22.4)	.81
Muscle Assessments			
Calf circumference, cm	31.0 [29.0-33.75]	34.0 [32.0-37.0]	.003
Mid-arm circumference, cm	26.0 [23.62-29.0]	27.0 [25.5-31.75]	.059
Waist circumference, cm	85.5 [74.5-98.75]	90.0 [81.50-100.0]	.11
Hip circumference, cm	92.5 [86.5-99.75]	99.0 [92.0-107.0]	.062
HGS, kg	22.55 [18.45-33.0]	37.5 [28.3-44.0]	<.001
Fat%	23.3 [15.35-29.9]	25.60 [16.8-32.6]	.47
FFMI, kg/m^2	17.55 [16.25-19.70]	18.90 [18.0-21.5]	.031
Phase angle	5.65 [4.45-6.52]	6.70 [6.0-9.30]	.001
Gait speed, m/s	0.96 [0.81-1.29]	1.24 [0.90-1.44]	.046
Rectus femoris muscle, mm	15.25 [13.37-18.1]	17.2 [14.9-19.35]	.047
Rectus femoris CsA, cm^2	6.27 [5.22-7.80]	7.63 [6.26-9.14]	.008
Medial head of gastrocnemius muscle, mm	14.30 [11.77-14.8]	17.0 [14.65-18.80]	<.001
Rectus abdominis muscle, mm	7.25 [5.15-8.9]	8.80 [7.3-9.7]	.027
External oblique muscle,mm	3.5 [3.02-4.5]	4.1 [3.5-4.7]	.14
Internal oblique muscle, mm	5.85 [4.5-7.72]	6.30 [5.00-7.90]	.58
Transversus abdominis muscle, mm	3.85 [3.32-4.35]	3.80 [3.30-4.60]	.99

Variables are given as n (%), mean ± SD, or median[25th-75th].
BMI, body mass index; HGS, handgrip strength; FFMI, fat-free mass index.

Table 4. Multivariable Logistic Regression Analysis of Possible Factors Associated with Sarcopenia

		OR	95% CI	*P*
Model 1	mTOR	1.25	0.44-3.56	.68
Model 2	Age	1.043	1.004-1.086	.033
Model 3	Age	1.051	1.001-1.105	.047

Model 1: Unadjusted model.
Model 2: Age, sex, use of mTOR.
Model 3: Age, sex, time since transplantation, proteinuria, kidney replacement therapy.

In the literature, the studies evaluating the effect of mTOR signaling on muscle mass and muscle wasting are mostly animal studies. In a study on inducible, muscle-specific Raptor and mTOR knocked-out mice, muscle histology and muscle strength were evaluated one and seven months following deletion. After 7 months, mTOR-knocked-out mice exhibited significant muscle weakness, mitochondrial dysfunction, and autophagy impairment. Whereas, the first month of this investigation indicated no change. According to the authors, this work demonstrated that mTOR signaling is essential for maintaining normal fiber innervation.[20] However, mTOR signaling is not the only mechanism engaged in the muscle innervation system[21]; the myostatin signaling pathway, ubiquitin-proteasome system, and protein degradation via the autophagy-lysosomal system are also implicated. The lack of difference between groups in our study may be due to the fact that blockage of a single pathway is insufficient to induce clinically meaningful changes in muscular strength or mass.

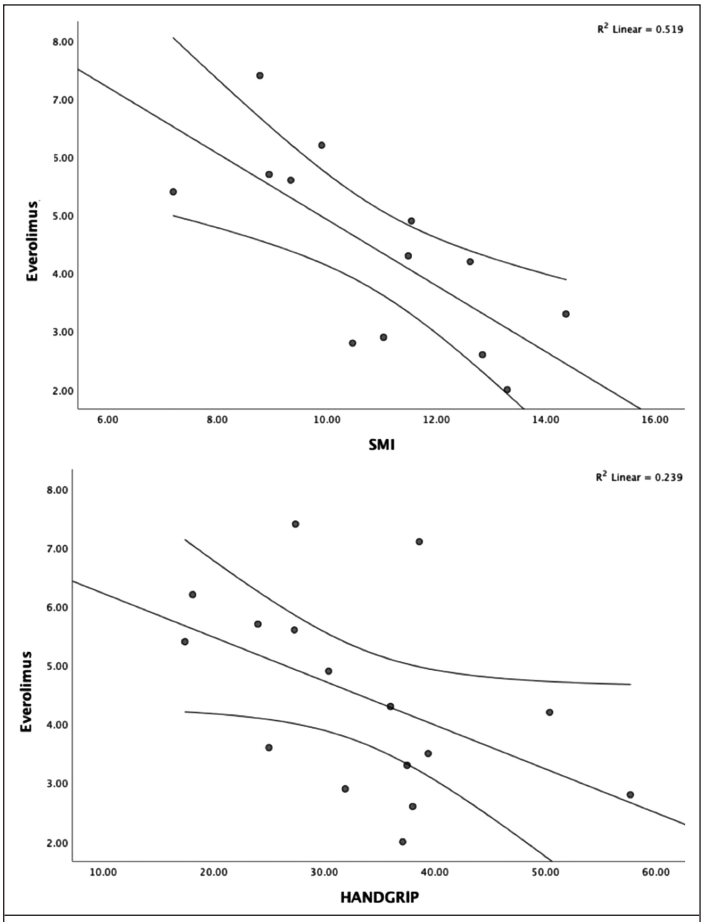

Figure 1. Correlation of Serum Everolimus Levels with Handgrip Strength and Skeletal Muscle Mass defined by BIA.

Another animal study investigated the potential of mTOR inhibitors to treat sarcopenia due to their longevity properties in mice[22] and their anti-aging effects on the immune system.[23] mTORC1 hyperactivation in aged muscle and partial inhibition of the mTOR pathway resulted in an improvement in muscle mass, albeit not in all muscle types.[24] mTOR inhibitors also had a favorable effect on muscles, which may have contributed to the absence of a difference between groups in our data. Consequently, the presence of both positive and negative effects could make it difficult to notice clinically distinct muscle loss. In clinical practice, there may be no difference between muscle loss and physical performance or strength for this reason exclusively.

In prior clinical studies, mTOR inhibitors were investigated mostly in cancer patients, although the results were contradictory. In a 2016 study by Gyawali et al[25], the authors reported that mTOR inhibitors had computed tomography-defined sarcopenic effects on cancer patients after six months of therapy. Similar to our findings, another study involving cancer patients found no difference between the sarcopenic group with cancer, the non-sarcopenic group with cancer, and the control (no cancer, no sarcopenia) group.[26] The present study differs from the aforementioned studies since sarcopenia was common in kidney failure and the mechanisms of sarcopenia and cachexia were different in cancer patients. We tried to assess muscle mass using both BIA and US, but no relationship between mTOR inhibitors and sarcopenia was observed in this specific patient population. Changes in molecular pathways often do not reflect the clinical manifestation in the patient. Numerous molecular pathways are implicated in the formation of sarcopenia, which complicates the real-world implications of molecular pathways.

Sarcopenia prevalence varies in KTRs between 11.1% and 49.6% according to the method that was utilized for the diagnosis of sarcopenia.[5,10,27-29] According to EWGSOP2 criteria, sarcopenia was identified in one out of every three KTRs in our study, similar to previous studies.

Sarcopenia in KTRs, as in older adults, is essential since it is closely related to adverse outcomes like increased hospitalization and mortality. In a recently conducted study from the TransplantLines Biobank, higher HGS levels were associated with lower mortality rates in KTRs.[30] Another study revealed that low muscle mass before kidney transplantation was also associated with hospital readmission in the one-year

post-transplant period.[31] The validity and reliability of the US in assessing muscle mass in older adults were investigated in previous studies, and it is widely used to define muscle quality and quantity.[32] Since assessment of muscle mass by BIA is not recommended in patients with KTRs to identify the higher-risk patients for adverse outcomes, we observed that muscle US may be the preferred tool for determining sarcopenia in this patient group. Our study first discovered that sarcopenic KTRs have lower muscle mass in lower extremity muscles and RA muscle by muscle US, which differs from previous research. Nevertheless, these findings need to be confirmed in prospective large population studies.

The novelty of our study resides in the observation of a noteworthy and robust correlation between the blood levels of everolimus and the metrics of HGS and SMI. Extensive literature has previously associated various medications, such as metformin, sulphonylureas, and statins, with the potential induction of sarcopenia, a condition characterized by muscle loss.[33] However, our investigation stands as the first to establish a discernible relationship between any pharmaceutical agent and the parameters of handgrip strength and muscle mass, with the sole exception of vitamin D, as documented in prior studies. It is worth noting that current literature research has demonstrated that diminished levels of vitamin D serve as a predictive factor for reduced grip strength and muscle mass.

We acknowledge some limitations of the study; the study was cross-sectional and there was no follow-up period with patients, the causal direction of the relationships could not be observed. Moreover, the study population is small, especially the number of those treated with the mTOR inhibitors because according to transplantation guidelines using a combination of a CNI and an antiproliferative agent, with or without corticosteroids, is recommended as maintenance therapy. Especially, it is suggested that tacrolimus be the first-line CNI used.[34] Our study has also had some strengths. Even though sarcopenia is commonly assessed in KTRs, studies evaluating mTOR inhibitors' association with sarcopenia and these frequently used medications in daily practice are rare. To the best of our knowledge, it is also the first study assessing the relationship between mTOR inhibitor treatment and sarcopenia in KTRs. Another strength is related to muscle mass evaluation. Most studies evaluate muscle mass using only one method; in our study, muscle mass is assessed both by BIA and muscle US. Muscle US could be used to identify sarcopenia as a valid, easy, and noninvasive method in KTRs.

To conclude, our findings indicate that there was no significant disparity in the prevalence of sarcopenia between the groups utilizing mTOR inhibitors and those not using them. This observation underscores the concept that alterations in molecular pathways may not consistently correspond to clinical outcomes in practical, real-world settings.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Hacettepe University (decision number: 2021/13-01).

Informed Consent: Written informed consent was obtained from patients who agreed to take part in the study.

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