



Effect of Converting From Calcineurin Inhibitor– to Sirolimus-Based Immunosuppressant Regimen on Breast Fibroadenoma Among Kidney Transplant Patients

Min Ji Kim^a, Byeong Woo Kim^a, Young Chul Yoon^b, Won Ik Seo^c, Young Mi Park^d, Sun Woo Kang^a, Tae Hee Kim^a, and Yeong Hoon Kim^{a,*}

^aDepartment of Internal Medicine, Busan Paik Hospital, Inje University, Busan, South Korea; ^bDepartment of Transplantation, Busan Paik Hospital, Inje University, Busan, South Korea; ^cDepartment of Urology, Busan Paik Hospital, Inje University, Busan, South Korea; and ^dDepartment of Radiology, Busan Paik Hospital, Inje University, Busan, South Korea

ABSTRACT

The incidence rate of breast fibroadenomas is higher among female kidney transplant (KT) patients treated using cyclosporine (CsA) for immunosuppression than in the general population. As such, there is an effort to convert immunosuppression from CsA or tacrolimus to sirolimus. Our aim was to assess the reversibility of a breast fibroadenoma after conversion in a small cohort of female KT recipients. This was an open-label, single-arm study including 128 female KT recipients, with a positive finding of a breast fibroadenoma in 15. Lesions were classified according to the Breast Imaging Reporting and Data System (BIRADS). Among these 15, a total of 7 converted from tacrolimus to sirolimus and 8 converted from CsA. We measured the change in BIRADS category and hormone and cytokine levels from baseline to 12 months after conversion. The primary outcome was progression or reversal of existing fibroadenomas at 12 months after conversion. Secondary outcomes were differences in hormone and cytokine levels. Conversion from CsA or tacrolimus to sirolimus had no significant effect on the BIRADS classification. However, conversion to sirolimus did produce a significant decrease in the level of transforming growth factor β cytokine, this level being closely associated with fibroadenomas. Conversion from a calcineurin inhibitor to sirolimus can block the progression of fibroadenomas. Further research is needed to confirm our results.

AFTER kidney transplant (KT), immunosuppressive agents are recommended to prevent graft rejection. Cyclosporine (CsA) and tacrolimus are 2 calcineurin inhibitors (CNIs) that are commonly used for immunosuppressant therapy. However, CNIs are associated with a number of adverse effects, the most common being impaired renal function, hypertension, increased risk for infections, hypertrichosis, fine tremor, impaired liver function, gingival hyperplasia, and breast fibroadenomas [1-10]. Regarding gingival hyperplasia, CsA acts directly on gingival fibroblasts, promoting both proliferation and collagen production [11]. A similar mechanism of action may underlie the development of breast fibroadenomas. Fibroadenomas are the most common benign solid lesions of the breast in young women. These are hormone-dependent neoplasms

that contain more estrogen receptors than the mammary lobule [10]. Fibroadenomas usually form during menarche and are most commonly found in women 15 to 25 years of age [12]. The rate of malignant transformation of these lesions is low, at <0.3% [13]. The incidence rate of fibroadenomas in the general population ranges between 7% and 13% [14], with an increase in the incidence rate among female KT patients receiving CsA having been reported [2-9].

This work was supported by Pfizer, Inc.

*Address correspondence to Yeong Hoon Kim, MD, Division of Nephrology, Inje, University College of Medicine, 75, Bokji-ro, Busanjin-gu, Busan, Republic of Korea. Tel: +82 51 890 6267. E-mail: yeonghnl@inje.ac.kr

When CNI toxicities, such as nephrotoxicity, develop, lowering the dose or withdrawal of CNIs is recommended. In cases in which CNI is withdrawn, sirolimus is usually introduced to maintain immunosuppressive therapy. However, the effect of converting from CsA to sirolimus on the progression of breast fibroadenomas has not been clarified. Therefore, our aim in this study was to evaluate the reversibility of breast fibroadenomas following conversion from a CsA- or tacrolimus-based to a sirolimus-based immunosuppressant regimen among a small cohort of female KT recipients.

MATERIALS AND METHODS

This is a cohort study of 128 women who underwent KT between August 1991 and December 2011 in a single transplantation center in Korea. This clinical trial was approved by the ethics committee of our center, and patients provided written informed consent prior to enrollment. Female renal transplant recipients older than 18 years who had been prescribed CsA or tacrolimus for >6 months were included. Patients who had experienced acute rejection in the past as well as those who were pregnant or needed hormone treatment at the time of conversion were excluded. Ultimately, 128 patients were enrolled into our open-label, single-arm study.

The features of fibroadenomas were examined by ultrasonography (USG) in all 128 patients. When a fibroadenoma was detected, we assessed changes in the baseline features of the mass at 12 months after immunosuppressant conversion. Patients agreed to use contraception from the time of baseline detection to the postconversion USG examination. Fibroadenomas were classified using the Breast Imaging Reporting and Data System (BIRADS), which is used in most countries where breast cancer screening has been implemented. The BIRADS 0 to 6 classification scores are defined as follows: 0, incomplete, requiring additional imaging; 1, normal; 2, typically benign; 3, probably benign; 4, suspicious abnormality; 5, highly suggestive of malignancy; and 6, histologically proven malignancy [15]. The normal follow-up is recommended for a breast mass with a BIRAD classification of 1 or 2, with a shorter follow-up for a grade 3 mass. A core biopsy was performed for histologic confirmation of diagnosis for a grade 4, 5, or 6 mass.

The protocol for conversion to a sirolimus-based regimen included a single loading dose (8.0 mg), provided between 4 and 24 hours after the last dose of CNI. From day 2, a daily dosage of 4.0 to 6.0 mg of sirolimus was administered until the dose of sirolimus reached a trough level. The sirolimus dose was then adjusted to 4.0 to 6.0 ng/mL, with the dose of mycophenolate and steroid continued as previously.

Hormones (including follicle-stimulating hormone, prolactin, estradiol, and progesterone) and cytokines (including transforming growth factor β [TGF β] and acidic fibroblast growth factor [FGF]) were measured at baseline and at the 12-month follow-up USG examination.

The primary outcome was progression or reversal of an existing fibroadenoma more than 1 year after conversion from a CNI- to a sirolimus-based immunosuppressant regimen. Secondary outcomes were the change in hormone and cytokine levels, from baseline to the 12-month follow-up. Change in all parameters

Table 1. Baseline Characteristics of All Patients

Characteristics	Overall
Age at KT, mean (SD), y	32 (10)
Age at enrollment, mean (SD), y	39.8 (8.9)
CNI before enrollment	
Tacrolimus, No. (%)	7 (46.7)
Duration, mo	84
Dose, μ g	4.17
Cyclosporine, No. (%)	8 (53.3)
Duration, mo	139.5
Dose, μ g	121.8

Abbreviations: CNI, calcineurin inhibitor; KT, kidney transplant.

from baseline was evaluated using a paired *t* test. All statistical analyses were performed using SPSS statistical software version 12.0 (IBM, Armonk, NY, United States), with a *P* value < .05 being significant.

RESULTS

From 1991 to 2011, a total of 128 women underwent KT, with a breast fibroadenoma and a BIRADS category ≥ 3 identified in 15. The mean age of the total group was 32 (SD, 10) years at the time of KT, with a mean age for the 15 patients with a breast fibroadenoma of 40 (SD, 9) years at the time of enrollment. Among these 15 patients, 7 (46.7%) had been using tacrolimus before conversion, and 8 (53.3%) had been using CsA (Table 1). Regarding the classification of fibroadenomas, 3 of the 15 patients had a BIRADS category 4A mass and underwent core needle biopsy for histologic confirmation of the diagnosis, with the remaining 12 having a category 3 mass. Pathologic results confirmed a benign lesion in all 3 cases, with a diagnosis of intraductal papilloma or fibrocystic change.

One year after conversion to the sirolimus-based regimen, the same radiologist examined the breast USG results for comparison of the features and BIRADS classification relative to baseline. One of the 3 patients with a category 4A mass developed severe gastrointestinal symptoms, including diarrhea and vomiting, requiring discontinuation of sirolimus and reversion to the CNI-based regimen. Regarding the other 2 patients with a category 4A mass, the classification remained stable on follow-up examination for 1 patient, with the other undergoing excisional biopsy to confirm the diagnosis of the category 4A mass. Among the remaining 12 patients with a category 3 mass at baseline, 3 developed diarrhea and vomiting after conversion to the sirolimus-based regimen, and 1 endometrial hyperplasia required surgical treatment. For these 3 patients, reversion to the CNI-based regimen was indicated. In 4 of the remaining 9 patients, the BIRADS category decreased from 3 (at baseline) to 2 (on follow-up) because of a slight reduction in the size of the fibroadenoma at 1 year after conversion to a sirolimus-based regimen (Table 2). Overall, however, there

Table 2. Change in Size of Breast Fibroadenoma at 1 Year After Conversion to Sirolimus-based Immunosuppressant

No.	Calcineurin Inhibitor	BIRADS Category	
		Baseline	After 1 Year
1	Cyclosporine	3	2
2	Cyclosporine	3	3
3	Tacrolimus	3	3
4	Cyclosporine	3	*
5	Cyclosporine	3	3
6	Tacrolimus	4A	4A
7	Tacrolimus	4A	3A
8	Cyclosporine	3	3
9	Tacrolimus	3	2
10	Tacrolimus	3	2
11	Cyclosporine	3	2
12	Tacrolimus	3	3
13	Cyclosporine	3	†
14	Cyclosporine	3	3
15	Tacrolimus	4A	*

Abbreviation: BIRADS, Breast Imaging Report and Data System.

*Dropout because of GI symptom.

†Dropout because of menorrhagia.

was no significant change in the shape or size of fibroadenomas after 1 year of modifying the immunosuppressant.

There were no significant changes in serum hormone levels from baseline to 12 months after conversion to a sirolimus-based regimen: estradiol, 87.49 pg/mL to 78.14 pg/mL ($P = .95$); follicle-stimulating hormone, 37.52 mIU/mL to 39.10 mIU/mL ($P = .95$); prolactin, 19.83 ng/mL to 20.02 ng/mL ($P = .97$); and progesterone, 1.46 ng/mL to 1.09 ng/mL ($P = .499$) (Table 3). Regarding cytokine levels, the mean level of transforming growth factor β (TGF β) decreased from 214.78 pg/mL at baseline to 196.62 pg/mL at the 12-month follow-up ($P = .05$). The mean level of acidic FGF also decreased from 15.86 pg/mL to 9.40 pg/mL, although this change was not significant ($P = .54$). Therefore, the use of CNIs is associated with an increase in the level of TGF β (Fig 1).

DISCUSSION

We identified a decrease in the level of TGF β cytokine. This decrease is clinically relevant as an increase in TGF β has been associated to fibroadenomas after the conversion from a CNI- to a sirolimus-based immunosuppressant regimen but with no effect on FGF. The mechanism

Table 3. Hormonal Change

Hormone	Baseline	12th Mo	P Value
Estradiol	87.49	78.14	.95
FSH	37.52	39.10	.95
Prolactin	19.83	20.02	.97
Progesterone	1.46	1.09	.499

Abbreviation: FSH, follicle-stimulating hormone.

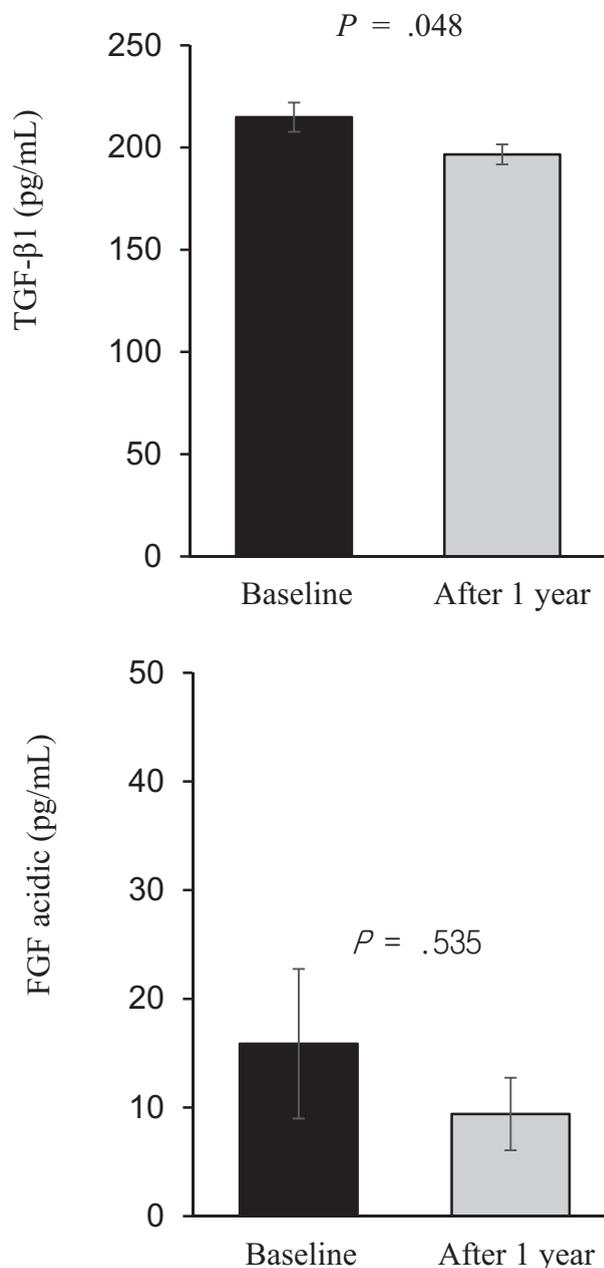


Fig 1. TGF β 1 and acidic FGF level at baseline and 1 year follow-up. FGF, fibroblast growth factor; TGF β 1, transforming growth factor β 1.

underlying this lowering in TGF β with sirolimus is unclear. Limited evidence from animal models has indicated that CsA itself may promote cancer progression by stimulating the production of TGF β . In vitro, CsA induces invasive behavior of nontransformed cells, with striking morphologic changes, promoting tumor growth in immunodeficient animals [16]. These CsA-induced effects can be prevented, both in vitro and in vivo, with the

administration of anti-TGF β antibodies. Additionally, an increase in proangiogenic effect has been reported with the use of CsA, resulting from an elevated expression of vascular endothelial growth factor [17–19]. Data from a case series provided some evidence of an increase in malignancy with the use of tacrolimus after KT [20]. This increase in malignancy likely results from an increase in TGF β levels [21], an effect that is clearly associated with tumor growth during CsA treatment. In our small cohort study, we identified a mild decrease in TGF β levels, which may have been associated with the decrease in BIRADS category of the mass from 3 to 2. As both of these lesion categories are benign, the clinical significance of a decrease in TGF β levels after conversion from CsA to sirolimus might not be clinically significant. However, it is important to note that the decrease in TGF β levels was associated with nonprogression of breast fibroadenomas.

There was no incidence of transplant rejection among these 15 patients at >29 months after conversion to sirolimus. Four patients had to discontinue the sirolimus-based regimen because of nonrenal adverse effects, including gastrointestinal symptoms in 3 of these patients (vomiting, diarrhea, and abdominal pain), with symptoms relieved by reversion to CNI from sirolimus. One patient developed menorrhagia and endometrial hyperplasia, requiring reversion to CNI before curettage. Of note, in rats, mammalian target of rapamycin (mTOR) inhibitors, which include sirolimus, have been shown to slow the progression of endometrial hyperplasia [22]; however, in our study, it was not clear whether the endometrial hyperplasia was associated with the use of sirolimus.

Previous studies have reported on the effectiveness of conversion from CsA or tacrolimus to sirolimus in preventing the progression of fibroadenomas [10]. Our results supported these findings, with conversion from CsA and tacrolimus to sirolimus preventing the progression of fibroadenomas. Therefore, it appears that both CsA and tacrolimus influence the progression of fibroadenomas, with a mild decrease in the level of TGF β after conversion of either CsA or tacrolimus to sirolimus.

There are several limitations in this study. First, because the cohort size is small, there is the possibility that the prevalence of fibroadenomas was underreported and misprocessed as a loss to follow-up. Second, the follow-up was short, at 12 months, and, therefore, we were unable to determine the effect of change over time or of the long-term exposure to sirolimus on fibroadenomas. Despite these limitations, we propose that conversion from a CNI to an mTOR inhibitor can block the progression of fibroadenomas. Therefore, we recommend early screening for fibroadenomas using USG in female KT recipients as well as conversion from a CNI- to an mTOR-based

immunosuppressant regimen to prevent the progression of breast fibroadenomas. However, considering the small size of our cohort and short-term follow-up, further research is needed to confirm our results.

REFERENCES

- [1] Von Graffenried B. Side effects of cyclosporine (Sandimmune) in renal transplant recipients and in patients with autoimmune diseases. *Transpl Proc* 1986;18:876–83.
- [2] Baildam A, Higgins R, Hurley E, et al. Cyclosporin A and multiple fibroadenomas of the breast. *Br J Surg* 1996;83:1755–7.
- [3] Kollias J, Gill PG, Leong ASY, Clarkson AR. Gynaecomastia presenting as fibroadenomatoid tumours of the breast in a renal transplant recipient associated with cyclosporin treatment. *Aust N Z J Surg* 1998;68:679–81.
- [4] Cyslak D, Carpenter PM. Breast imaging case of the day. *Radiographics* 1999;19:S80–3.
- [5] Muttarak M, Peh WC, Chaiwun B, Lumlertgul D. Multiple bilateral giant fibroadenomas associated with cyclosporine A therapy in a renal transplant recipient. *Australas Radiol* 2001;45:517–9.
- [6] Weinstein SP, Orel SG, Collazzo L, Conant EF, Lawton TJ, Czerniecki B. Cyclosporin A-induced fibroadenomas of the breast: report of 5 cases. *Radiology* 2001;220:465–8.
- [7] Caetano Stefenon C, de Oliveira Lima R, Gualandi Murad AL. Cyclosporine and the development of multiple mammary nodules. *Breast J* 2002;8:177–9.
- [8] Sangthawan P, Fox J, Atkins RC, Kerr PG. Increased incidence of benign breast disease in female renal transplant patients receiving cyclosporin. *ANZ J Surg* 2002;72:222–5.
- [9] Balal M, Seyrek N, Karayaylali I, Paydas S. Report of a renal transplanted patient with fibroadenoma occurring in a short time. *Transplant Proc* 2003;35:1408–9.
- [10] Iaria G, Pisani F, De Luca L, et al. Prospective study of switch from cyclosporine to tacrolimus for fibroadenomas of the breast in kidney transplantation. *Transplant Proc* 2010;42:1169–70.
- [11] Schincaglia G, Forniti F, Cavallini R, et al. Cyclosporin-A increases type I procollagen production and mRNA level in human gingival fibroblasts in vitro. *J Oral Pathol Med* 1992;21:181–5.
- [12] Engels EA, Pfeiffer RM, Fraumeni JF, et al. Spectrum of cancer risk among US solid organ transplant recipients. *JAMA* 2011;306:1891–901.
- [13] Sklair-Levy M, Sella T, Alweiss T, Craciun I, Libson E, Mally B. Incidence and management of complex fibroadenomas. *AJR Am J Roentgenol* 2008;190:214–8.
- [14] Greenberg R, Skornick Y, Kaplan O. Management of breast fibroadenomas. *J Gen Intern Med* 1998;13:640–5.
- [15] Balleyguier C, Ayadi S, Van Nguyen K, Vanel D, Dromain C, Sigal R. BIRADS classification in mammography. *Eur J Radiol* 2007;61:192–4.
- [16] Hojo M, Morimoto T, Maluccio M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 1999;397:530.
- [17] Guba M, Von Breitenbuch P, Steinbauer M, et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002;8:128.
- [18] Shihab FS, Bennett WM, Isaac J, Yi H, Andoh TF. Nitric oxide modulates vascular endothelial growth factor and receptors

in chronic cyclosporine nephrotoxicity. *Kidney Int* 2003;63:522–33.

[19] Guba M, Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. *Transplantation* 2004;77:1777–82.

[20] Imao T, Ichimaru N, Takahara S, et al. Risk factors for malignancy in Japanese renal transplant recipients. *Cancer* 2007;109:2109–15.

[21] Maluccio M, Sharma V, Lagman M, et al. Tacrolimus enhances transforming growth factor- β 1 expression and promotes tumor progression. *Transplantation* 2003;76:597–602.

[22] Milam MR, Celestino J, Wu W, et al. Reduced progression of endometrial hyperplasia with oral mTOR inhibition in the Pten heterozygote murine model. *Am J Obstet Gynecol* 2007;196:247.e1–5.