Can Lipofuscin Deposition on Renal Allograft Tubular Epithelium Be a Surrogate Marker for Kidney Allograft Aging?

Yuki Kawaguchi[a], Hideyo Oguchi[a,*], Tetuo Mikami[b], Yutaka Yamaguchi[c], Yasushi Ohashi[d], Takeshi Kawamura[d], Masaki Muramatsu[a], Yoshihiro Itabashi[a], Kazunobu Shinoda[a], Yoji Hyodo[a], Yusuke Takahashi[a], Hiroka Onishi[a], Taichi Arai[a], Yuko Hamasaki[e], Kazutoshi Shibuya[f], Seiichiro Shishido[a], and Ken Sakai[a]

[a]Department of Nephrology, Toho University Faculty of Medicine, Tokyo, Japan; [b]Department of Pathology, Toho University Faculty of Medicine, Tokyo, Japan; [c]Yamaguchi's Pathology Laboratory, Chiba, Japan; [d]Department of Nephrology, Sakura Medical Center, Toho University, Chiba, Japan; [e]Department of Pediatric Nephrology, Toho University Faculty of Medicine, Tokyo, Japan; and [f]Department of Surgical Pathology, Toho University Faculty of Medicine, Tokyo, Japan

ABSTRACT

Background. Lipofuscin is an indicator of aging. We examined the clinicopathologic significance of lipofuscin deposition in the renal tubules of renal allografts.

Method. We analyzed allograft biopsy specimens from living kidney transplantations from January to December 2015. For controls, we analyzed native kidney biopsy specimens obtained from January 2015 to December 2016. We identified granules with a yellow-to-tan shade in renal tubules as lipofuscin.

Results. The donor age at transplantation was significantly older in lipofuscin deposition biopsy specimens than in those without, whereas the time after transplantation age was not different between the 2 groups with renal allografts. In native kidney biopsies, age at biopsy was significantly older in lipofuscin deposition biopsy specimens than in those without. We compared "massive lipofuscin deposition," defined as lipofuscin deposition on both sides of 3 or more renal tubules, and donor-age matched control allograft biopsies without lipofuscin deposition. Comparing these 2 groups, recipient age at transplantation was significantly older in the massive lipofuscin deposition group.

Conclusion. Lipofuscin deposition on tubular epithelium is not a surrogate marker of aging of kidneys allografts, although lipofuscin deposition was significantly greater in older tissues from native kidneys. The older age of recipients may be associated with massive lipofuscin deposition in renal allografts.

LIPOFUSCIN is reported to be a yellow-brown material that progressively accumulates over time in the lysosomes of postmitotic cells [1]. Its accumulation within postmitotic cells is a recognized hallmark of aging [2]. Lipofuscin was reported to accumulate in the lysosomes of neurons and cardiac myocytes [1], and its deposition promoted the development of macular degeneration [2]. Furthermore, lipofuscin increased with age in human kidneys and was present in tubular cells but not glomeruli [3]. Routine observation often reveals lipofuscin deposition in the renal allograft tubular epithelium (Fig 1A); however, lipofuscin deposition in renal allografts has rarely been reported.

Pathological surrogate markers of native kidney and allografts have been reported previously. One study reported that p16 (INK4a) and p27 (Kip1) CDKI genes increase in aging native kidney [4], and another report stated that Telomere length was significantly lower in tubular epithelial cells expressing SA-beta-Gal than in cells without expressing in renal allografts [5]. However,

*Address correspondence to Hideyo Oguchi, Department of Nephrology, Toho University Faculty of Medicine, 6-11-1, Omori-Nishi, Ota-ku, Tokyo 143-8541, Japan. Tel: +81-3-3762-4151; Fax: +81-3-5471-3056. E-mail: 302211oh@med.toho-u.ac.jp

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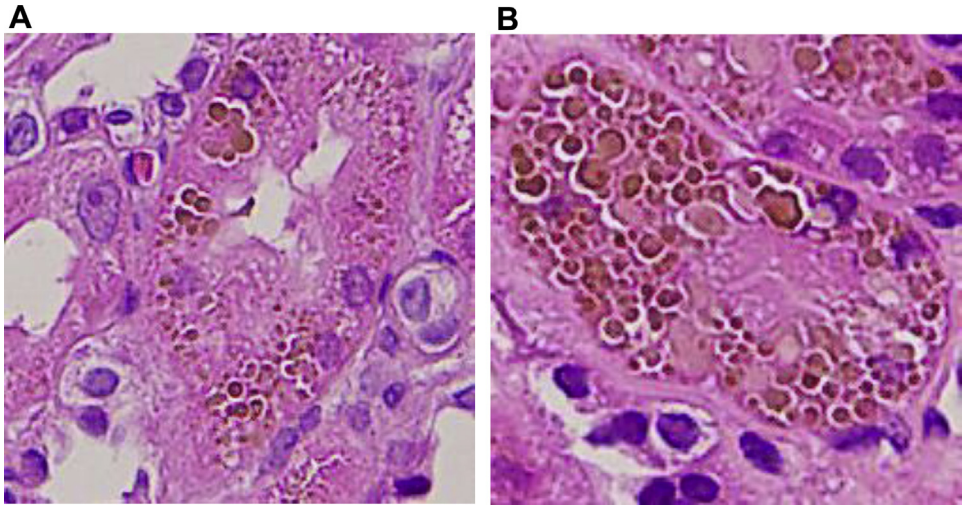

Fig 1. **(A)** Representative figure of lipofuscin. **(B)** Representative figure of massive lipofuscin deposition defined by lipofuscin deposition on both sides of tubules and 3 or more renal tubules.

whether lipofuscin deposition in the tubular cells of renal allografts is a surrogate marker for kidney graft aging has not been reported, and the clinicopathologic significance of lipofuscin deposition on renal allograft tubular epithelium is unknown.

The aim of this study was to examine lipofuscin deposition in the renal allograft tubular epithelium as a surrogate marker for kidney allograft aging and to analyze the clinicopathologic significance of lipofuscin deposition on renal allograft tubular epithelium.

MATERIALS AND METHODS

At our institution, protocol biopsies were routinely performed at 1 hour, 3 months, 7 years, and in some recipients at 10 years after kidney transplantation. We analyzed allograft protocol or episode biopsy specimens performed at our hospital from January to December 2015 from living kidney transplantations. As a control, we analyzed native kidney biopsy specimens from January 2015 to December 2016. We evaluated 91 native biopsy specimens obtained from 91 patients and 260 transplant biopsy specimens obtained from 201 recipients.

For light microscopy in our hospital, hematoxylin and eosin, periodic acid-Schiff, periodic acid-methenamine silver, and Masson trichrome stains are routinely performed for renal allografts and native kidneys. We identified granules with a yellow-to-tan shade in renal tubules as lipofuscin (representative image shown in Fig 1A). Histological scores were based on the Banff classification [6,7]. Massive lipofuscin deposition (Fig 1B) was defined as "lipofuscin deposition on both sides of the proximal tubule and three or more renal tubules." We compared the massive lipofuscin deposition group to donor-age matched biopsy specimens lacking lipofuscin deposition. Both univariate and multivariate analysis were performed using a logistic regression model.

All statistical analyses were performed with SPSS software version 23.0 for Windows (IBM Japan, Tokyo, Japan). A P value of $< .05$ was regarded to indicate statistically significant differences. We compared variables using the χ^2, Fisher's exact test, the Mann-Whitney U test, and Student t test. All data are presented as the number (%) or mean ± standard deviation or median and range of distribution for continuous variables. Our study was approved by the Ethics Committee of Toho University, Omori Hospital (approval number M18136).

RESULTS

Clinical Background and Prevalence of Lipofuscin Deposition Between Allograft and Native Biopsies

Table 1 shows the clinical background and prevalence of deposition between allograft and native biopsies. In renal allograft biopsy specimens, donor age at transplantation was 51.7 ± 12.4 years. The duration after transplantation was 31.5 ± 43.0 months, male/female ratio was 157/103, and prevalence of lipofuscin deposition was 58.8%. The prevalence of diabetes treatment was 25.8%, prevalence of hypertension treatment was 65.4%, and the body mass index was 20.2 ± 4.5 in allograft kidney biopsies. The original disease of allograft kidney biopsies was shown in the Table 1. In native kidney biopsy specimens, age at native biopsy was 52.5 ± 19.5 years, male/female ratio was 44/47, and the prevalence of lipofuscin deposition was 69.2%. The prevalence of diabetes treatment was 4.4%, the prevalence of hypertension treatment was 28.6%, and the body mass index was 23.3 ± 3.6 in native kidney biopsies. The diagnosis of native kidney biopsies was described in the Table 1. In renal allografts, lipofuscin deposition was localized in the proximal tubules of 84 biopsy specimens and in tubules other than proximal tubules in 69 biopsy specimens. In native biopsies, lipofuscin deposition was localized in the proximal tubules of 30 biopsy specimens and in tubules other than proximal tubules in 33 biopsy specimens.

Table 1. Clinical Background and Prevalence of Deposition Between Allograft and Native Biopsies

	Allograft Kidney Biopsies (n = 260)	Native Kidney Biopsies (n = 91)
Age at the native biopsy (years)	-	52.5 ± 19.5
Donor age at transplantation (years)	51.7 ± 12.4	-
Duration after transplantation (months)	31.5 ± 43.0	-
Recipient age at transplantation (years)	32.2 ± 20.4	-
Male/female, n	157/103	44/47
Prevalence of lipofuscin deposition, n (%)	153 (58.8)	63 (69.2)
Diabetes treatment, n (%)	67 (25.8)	4 (4.4)
Hypertension treatment, n (%)	170 (65.4)	26 (28.6)
BMI	20.2 ± 4.5	23.3 ± 3.6
Original disease of allograft kidney biopsies or diagnosis of native kidney biopsies		
Hypoplastic/dysplastic kidney	42 (16.2)	-
IgA nephropathy	34 (13.1)	32 (35.2)
Diabetic nephropathy	23 (8.8)	4 (4.4)
Focal segmental glomerulosclerosis	16 (6.2)	1 (1.1)
Nephrosclerosis	10 (3.8)	-
Lupus nephritis	5 (1.9)	9 (9.9)
Membranoproliferative glomerulonephritis	5 (1.9)	2 (2.2)
Polycystic kidney disease	4 (1.5)	-
Alport syndrome	2 (0.8)	-
Other	119 (45.8)	43 (47.3)

Abbreviation: BMI, body mass index;

Donor Age, Time After Transplantation in Kidney Allografts, and Age at Time of Native Biopsy in the Presence or Absence of Lipofuscin Deposition

Figure 2A shows the donor age in the presence and absence of lipofuscin deposition. Donor age was significantly older in the presence of lipofuscin deposition group compared with the absence of lipofuscin deposition group (median, [range]; 56 [26–80] vs 49 [20–80], $P = .027$, respectively). Figure 2B shows the time after transplantation in the presence or absence of lipofuscin deposition. The time after transplantation age was not different between both groups (12 [0–203] vs 11 [0–232], $P = .430$, respectively). Figure 2C shows the age at the time of native biopsy was significantly older in the presence of lipofuscin deposition group compared with the absence of lipofuscin deposition group (58 [16–84] vs 44 [20–81], $P = .023$, respectively).

Clinicopathologic Characteristics Between the Massive Lipofuscin Deposition Group and Donor-Age Matched Group Without Lipofuscin Deposition

Massive lipofuscin deposition was observed only in the proximal tubules. Table 2 shows the clinicopathologic characteristics between the massive lipofuscin deposition group and the donor-age matched group without lipofuscin deposition. Recipient age was significantly older in the massive lipofuscin deposition group compared with the donor-age matched group without lipofuscin deposition. Diabetes treatment were more prevalent with the donor-age matched group without lipofuscin deposition. There were no significant differences in sex prevalence; total ischemic time; cold ischemic time; recipient age at transplantation; duration after transplantation; tacrolimus or cyclosporine trough levels; serum creatinine at the time of biopsy; history of acute rejection; and Banff scores for t, i, ct, ci, ah, and aah. To identify risk factors associated with massive lipofuscin deposition, multivariate analyses were performed using these related covariates, including donor age, in all subjects (n = 122). As shown in Table 3, recipient age, but not donor age and prevalence of diabetes treatment, was an independent risk factor for massive lipofuscin deposition.

DISCUSSION

In our study, there was no difference in time after transplantation between the presence of lipofuscin deposition group and absence of lipofuscin deposition group, although donor age was significantly older in the presence of lipofuscin deposition group with renal allografts. For native kidney biopsies, age at biopsy was significantly older in the presence of lipofuscin group. Lipofuscin deposition in the tubular epithelium was not a surrogate marker of aging in kidneys allografts. The older age of recipients may be associated with massive lipofuscin deposition in renal allografts.

Lipofuscin was reported to increase with age in both human kidneys and rat models by previous report [3,8]. We

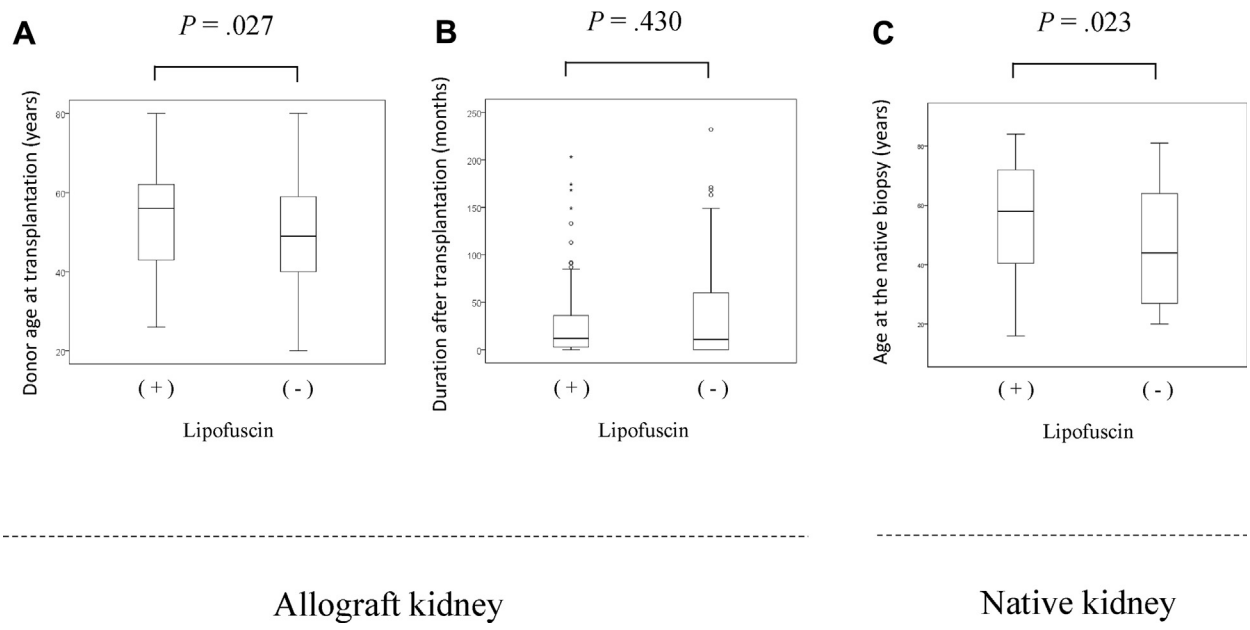

Fig 2. **(A)** Donor age in the presence or absence of lipofuscin deposition. Donor age was significantly older in the presence of lipofuscin deposition group compared with the absence of lipofuscin deposition group. **(B)** The time after transplantation in the presence or absence of lipofuscin deposition. The time after transplantation age was not different between groups. **(C)** Age at the time of native biopsy in the presence or absence of lipofuscin deposition was significantly older in presence of lipofuscin deposition group compared with the absence of lipofuscin deposition group.

also confirmed that donor age at renal allograft biopsy and age at native biopsy were significantly older (Figs 2A and 2C) in the presence of lipofuscin deposition group than in those without, whereas the time after transplantation age was not different between the 2 groups with renal allografts (Fig 2B). Furthermore, we defined massive lipofuscin deposition as lipofuscin deposition on both sides of 3 or more renal tubules, and massive lipofuscin deposition was

Table 2. Clinicopathologic Characteristics Between Massive Lipofuscin Deposition Group and Donor-Age Matched Group Without Lipofuscin Deposition

	Massive Lipofuscin Deposition Group (n = 15)	Donor Age Matched Group Without Lipofuscin Deposition (n = 16)	*P* Value
Male/female, n	8/7	11/5	.379
Total ischemic time (minute)	72.9 ± 20.8	87.7 ± 45.0	.737
Warm ischemic time (minute)	3.5 ± 1.3	4.1 ± 1.6	.317
Donor age at transplantation (years)	58.5 ± 10.5	56.5 ± 9.2	.569
Recipient age at transplantation (years)	48.5 ± 11.3	25.3 ± 11.8	<.01
Duration after transplantation (months)	48.0 ± 41.7	40.2 ± 39.9	.427
Tacrolimus trough level (ng/mL)	7.4 ± 2.7 (n = 5)	5.4 ± 1.6 (n = 10)	.141
Cyclosporine trough level (ng/mL)	86.3 ± 35.4 (n = 10)	139.2 ± 106.3 (n = 6)	.163
Serum Cr (mg/dL) at the time of biopsy	1.46 ± 0.35	1.36 ± 0.58	.558
Diabetes treatment, n	8/15	3/16	.044
The history of acute rejection, n	4/15	3/16	.461
Banff score			
t score	0.40 ± 0.83	0.56 ± 0.63	.24
i score	0.40 ± 0.51	0.44 ± 0.51	.835
ct score	1.47 ± 0.64	1.13 ± 0.50	.081
ci score	1.07 ± 0.80	0.69 ± 0.70	.175
ah score	1.87 ± 1.19	1.75 ± 1.00	.71
aah score	1.13 ± 1.30	1.19 ± 1.11	.833

Abbreviation: Cr, creatinine.

Table 3. Univariate and Multivariate Analysis of Factors Associated With Massive Lipofuscin Deposition

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Donor age at transplantation (years)	1.06 (1.01–1.11)	.016	1.02 (0.97–1.08)	.478
Recipient age at transplantation (years)	1.06 (1.02–1.09)	.002	1.04 (1.00–1.09)	.042
Diabetes treatment, n	3.95 (1.30–12.01)	.015	1.70 (0.48–6.00)	.409

Abbreviations: CI, confidence interval; OR, odds ratio.

observed only in the proximal tubules. A previous study reported lipofuscin deposition was increased with aging, and that lipofuscin was localized in mainly proximal tubules in a rat nontransplanted model [8]. We analyzed the clinicopathologic characteristics between the massive lipofuscin deposition group and donor-age matched group without lipofuscin deposition to investigate the potential reasons for deposition other than donor age.

Our data demonstrated that the duration after transplantation was not significantly different between the massive lipofuscin deposition group and donor-age matched group without lipofuscin deposition (Table 2). This suggests that lipofuscin deposition in the renal allograft tubular epithelium is not a surrogate marker for kidney allograft aging. A previous report suggested that lipofuscin was a waste material resulting from the insufficient digestion of oxidatively damaged macromolecules by autophagy in lysosomes [9]. Our data showed that ischemic time and Banff scores were not different between the 2 groups (Table 2). In the kidney allografts, differences in the function of autophagy may be involved in lipofuscin deposition, and a molecular approach will be required to demonstrate this.

Interestingly, recipient age was significantly older in the massive lipofuscin deposition group compared with the donor-age matched group without lipofuscin deposition (Table 2). A previous study reported that a minority of extra renal recipient cells were incorporated into the peritubular endothelium in a kidney transplanted rat model of renal endothelial cell injury [10]. Another report suggested that lipofuscin deposition is caused by an age-related decline in lysosomal degradation [1]. We speculate that decline in lysosomal degradation of renal tubular epithelium derived from older recipients by chimerism may cause lipofuscin deposition, while the maintained function of lysosome of renal tubular epithelium derived from younger recipients by chimerism may cause less lipofuscin deposition.

A major limitation of this study was its cross-sectional approach. A previous study suggested that lipofuscin may have a negative effect by preventing cellular renewal and advancing the accumulation of damaged cellular constituents [9]. Prospective studies are needed to clarify the effect on clinicopathologic graft outcome, and a molecular study is required to elucidate the pathogenesis of lipofuscin deposition.

CONCLUSION

Lipofuscin deposition on tubular epithelium is not a surrogate marker of aging in kidneys allografts, although lipofuscin deposition was significantly greater in older tissues of native kidneys. The older age of recipients may be involved in lipofuscin deposition in renal allografts. Further studies using a molecular approach are needed to clarify the clinicopathologic significance of lipofuscin deposition in renal allograft tubular epithelium.

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