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Abbreviations: BMP: bone morphogenic protein, EMT: epithelial-mesenchymal transition, EPO: erythropoietin, FSP: fibroblast specific protein, GSK: glycogen synthase kinase, HGF: hepatocyte growth factor, ILK: integrin-linked kinase, MET: mesenchymal-epithelial transition, MMP: metalloproteinase, PI3K: phosphoinositide-3-kinase, SMA: smooth muscle actin, TEC: tubular epithelial cell, TGF: transforming growth factor

Key Words: TGF-beta, EMT, renal fibrosis, Snail, Fibroblast, Tubular Epithelial Cell, Review

Send correspondence to: Masayuki Iwano,1st Department of Internal Medicine, Nara Medical University, 840 Shijo, Kashihara, Nara 634-8522, Japan, Tel: 81-744-22-3051, Fax: 81-744-22-9726, E-mail miwano@naramed-u.ac.jp

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糖尿病性細小血管症（第2版）

—発症・進展制御の最前線—

II. 糖尿病性腎症

検査マーカーとその意義

尿中ポドサイト

山口通雅　　岩野正之　　斎藤能彦

検査マーカーとその意義

尿中ポドサイト

Urinary podocytes

山口通雅　岩野正之　斎藤能彦

Key words：尿中ポドサイト，糖尿病性腎症，epithelial mesenchymal transition，fibroblast specific protein 1

はじめに

ポドサイトは糸球体を構成する細胞の一つであり，タンパク尿を防ぐためのバリアー機能や糸球体の構造維持に重要な役割を果たすことが知られている．近年，様々な腎疾患でポドサイト数の減少がタンパク尿と糸球体硬化を誘導することが明らかとなり，糸球体疾患の重要な進展機序として注目されている[1-4]．ポドサイト数の減少には，ポドサイトのアポトーシスや糸球体基底膜からの剥離が関与すると考えられている．Haraら[5]は，活動性のIgA腎症患者の尿中でポドサイトが検出されることを初めて報告した．更に，Nakamuraら[6]は顕性腎症期の糖尿病患者の尿中にはポドサイトが検出され，尿中ポドサイト数は疾患活動性のマーカとして有用であるとことを報告した．高血糖，糸球体過剰濾過，TGF-β[7]，およびangiotensin II[8]などの刺激がポドサイトに加わることにより，ポドサイトにアポトーシスが誘導されることが報告されている．一方，Vogelmannら[9]はループス腎炎などの活動性腎疾患患者の尿中に排泄されたポドサイトが培養液中で増殖可能であることを報告し，ポドサイトの糸球体基底膜からの剥離にはアポトーシス以外の機序が関与することを提唱した．加えて，著者ら[10]は糖尿病性腎症患者の糸球体ポドサイトおよび尿中ポドサイトにepithelial mesenchymal transition（EMT）（上皮細胞がその特異形質を失い，代わりに間葉系細胞の形質を発現する現象）の鍵分子であるfibroblast specific protein 1（FSP1）の発現が誘導されることから，ポドサイトがEMTにより運動能を獲得し，糸球体基底膜から剥離するという新しい機序を提唱した．FSP1はカルシウム結合タンパクであるS100ファミリーに属し，線維芽細胞や転移性癌細胞に発現し，細胞運動能に関与することが報告されている[11]．

本稿では，糖尿病性腎症における尿中ポドサイトの臨床的意義や糖尿病性腎症進展におけるポドサイトのEMTの関与について，自験例を中心に概説する．

1. 糖尿病性腎症における尿中ポドサイト

Nakamuraら[6]は抗ポドカリキシン抗体を用いた免疫蛍光染色により，糖尿病患者の尿中にポドサイトが排泄されることを報告した．顕性腎症期の患者では，腎症前期や早期腎症期の患者と比較して尿中ポドサイト数が増加していることから，尿中ポドサイト数は糖尿病性腎症の疾患活動性マーカとして有用であると考えられる．

著者ら[10]は当科通院中の2型糖尿病患者109

Yukinari Yamaguchi, Masayuki Iwano, Yoshihiko Saito: First Department of Internal Medicine, Nara Medical University　奈良県立医科大学 第1内科

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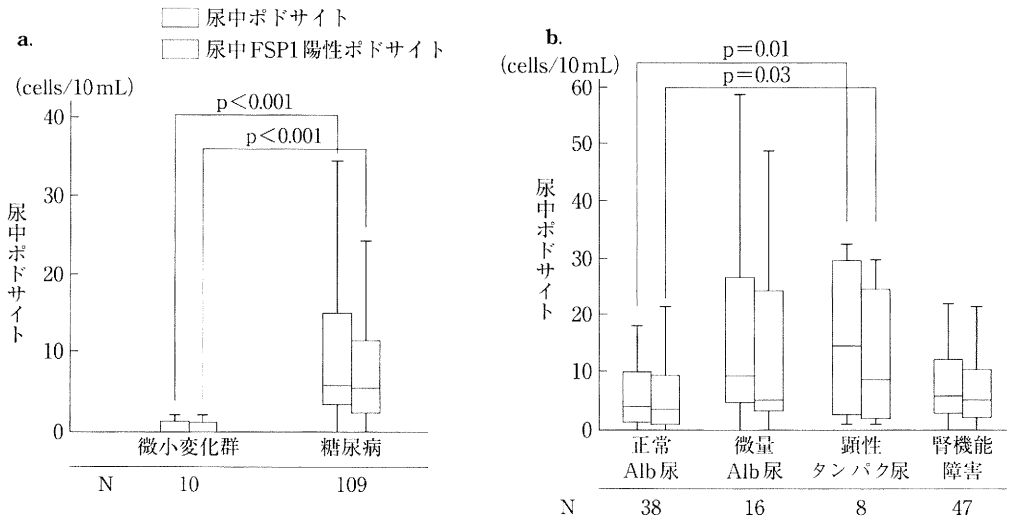

図1　微小変化群と糖尿病性腎症における尿中ポドサイト（文献[10]より改変）

a: 微小変化群と糖尿病群における尿中ポドサイト数(□)と尿中FSP1陽性ポドサイト数(■).

b: 糖尿病群における尿中ポドサイト数(□)と尿中FSP1陽性ポドサイト数(■).

糖尿病群は尿中アルブミン排泄と推定糸球体濾過量に基づき4群へ分類(正常Alb尿群，微量Alb尿群，顕性タンパク尿群，腎機能障害群).

例を対象に，尿中ポドサイト数および尿中FSP1陽性ポドサイト数について検討した．最初に，腎生検所見で糸球体に異常が認められなかった微小変化群10例と糖尿病群109例の尿中ポドサイト数について比較した．糖尿病群では，有意に尿中ポドサイト数の増加が認められた．更に，尿中に排泄されたポドサイトの8割以上がEMTの鍵分子であるFSP1を発現することから，ポドサイトの糸球体基底膜からの剥離にはポドサイトのEMTが関与することが示唆された(**図1-a**)．つぎに，尿中アルブミン排泄量と腎機能に基づき，糖尿病患者を4群(正常アルブミン尿群，微量アルブミン尿群，顕性タンパク尿群，腎機能障害群)に分類し，尿中ポドサイト数について検討した．正常アルブミン尿群と比較して顕性タンパク尿群では，尿中ポドサイト数が増加していた．すべての群において尿中へ排泄されたポドサイトの8割以上がFSP1を発現していた．一方，尿中ポドサイト数は腎機能障害群において減少していたが，このポドサイト数の減少は腎症進行期には糸球体ポドサイト自体が既に減少していることを反映すると考えられた(**図1-b**)．

2. 糖尿病性腎症における糸球体内FSP1発現

著者らは糖尿病性腎症における糸球体内FSP1発現の局在を検討するため，腎症患者の腎生検凍結切片を用いて，ポドサイトマーカであるシナプトポジンとFSP1に対する免疫蛍光2重染色を実施した．糖尿病性腎症患者の糸球体内ではメサンギウム細胞や内皮細胞におけるFSP1の発現は認められず，主にポドサイトでFSP1の発現が確認された．

つぎに，著者らは糖尿病性腎症における糸球体病変の重症度とポドサイトのFSP1発現との関連について検討した．糖尿病性腎症の糸球体病変を5群(G1: 局所的なメサンギウム増生を認める糸球体，G2: びまん性メサンギウム増生を認める糸球体，G3: 1個の結節性病変を認める糸球体，G4: 数個の結節性病変を認める糸球体，G5: 球状硬化を認める糸球体)に分類し(**図2-a**)，各群における糸球体1断面あたりのポドサイト数とFSP1陽性ポドサイト数を計測した

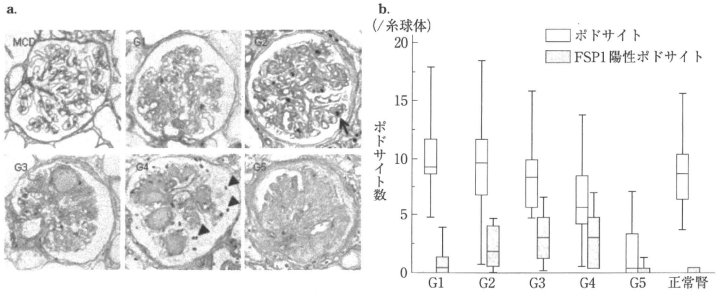

図2 糖尿病性腎症糸球体におけるポドサイト数とFSP1陽性ポドサイト数(文献[10]より改変)

a: 微小変化群と糖尿病性腎症における4型コラーゲンとFSP1の免疫2重染色像．矢印：FSP1陽性ポドサイトは糸球体基底膜の外側に位置する黒色の細胞として観察される．矢頭：G4の糸球体ではFSP1陽性ポドサイトが糸球体基底膜から剥離している像が観察される．

b: 腎生検組織における糸球体1断面あたりのポドサイト数(□)とFSP1陽性ポドサイト数(□)．

(**図2-b**)．糸球体1断面あたりのポドサイト数は，糸球体病変が進行するにつれて，すなわちメサンギウム基質と結節性病変が増加するにつれて，減少した．一方，FSP1陽性ポドサイト数は，メサンギウム基質と結節性病変が増加するにつれて増加したが，球状硬化を示す糸球体では著明に減少していた．更に興味深いことに，結節性病変が多数認められる糸球体(G4)ではFSP1陽性ポドサイトが糸球体基底膜から多数剥離している像が確認された(**図2-a**)．以上の成績から，①糸球体病変の進展とともにFSP1陽性ポドサイト数が増加する，②結節性病変が多数出現する腎症進行期に，糸球体基底膜からFSP1陽性ポドサイトが剥離する，③ポドサイトが剥離した糸球体は球状硬化に至る，という進展機序が示唆された．

3. 糖尿病性腎症進展の機序

糖尿病性腎症では糸球体内TGF-β1の発現が亢進していることが報告されている[12]．著者らは，①培養ポドサイト細胞株を用いた検討から，TGF-β1刺激により強力なEMT誘導転写因子であるSnail1のmRNA発現が著明に亢進すること，②糖尿病性腎症患者の糸球体ポドサイトでSnail1の局在が確認されること，③糖尿病性腎症進行期(G3-G4)の糸球体ポドサイトで上皮細胞マーカであるzonula occludens-1(ZO-1)の発現低下が認められることを確認した．以上の成績から，糖尿病性腎症の進展機序にはポドサイトのEMTが深く関与することが示唆された(**図3**)．

おわりに

糖尿病性腎症における尿中ポドサイトと腎症進展におけるポドサイトのEMTの意義について概説した．糖尿病性腎症における尿中FSP1陽性ポドサイトや腎生検組織におけるFSP1陽性ポドサイトは腎症進展のメカニズムを解明する鍵となり，疾患活動性を予測する新しい臨床マーカとして期待される．

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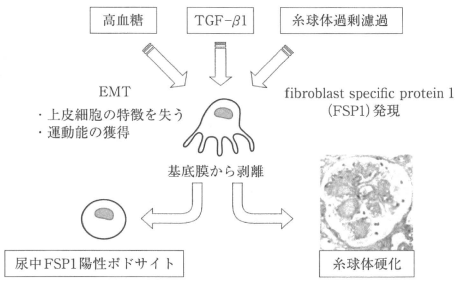

図3　糖尿病性腎症進展の機序

高血糖，TGF-β1，糸球体過剰濾過，およびangiotensin IIなどの刺激がポドサイトにEMTを誘導し，ポドサイトが糸球体基底膜から剥離する．その結果，尿中にFSP1陽性ポドサイトが排泄され，糸球体硬化が誘導される．

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Therapeutic Apheresis and Dialysis

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Effectiveness of Weekly Percutaneous Maxacalcitol Injection Therapy in Patients With Secondary Hyperparathyroidism

Atsushi Saito,[1] Yuji Matsumoto,[2] Yuhta Oyama,[3] Mitsuhiro Asaka,[4] and Hitoshi Yokoyama[4]

[1]Department of Nephrology, Kanazawa Medical University Himi Municipal Hospital, Toyama, [2]Department of Surgery, Honma Hospital, and [3]Department of Medicine, Nihonkai General Hospital, Yamagata, and [4]Division of Nephrology, Kanazawa Medical University, Ishikawa, Japan

Abstract: Percutaneous injection therapy with vitamin D has been applied in the treatment of hyperparathyroidism (HPT); however, the application of percutaneous injection therapy with vitamin D lacks established guidelines regarding the volume of injected solution and the frequency of injection. We have developed an outpatient treatment regimen using percutaneous maxacalcitol injection therapy (PMIT) on a weekly basis for 4–6 weeks following dialysis without major complications. Intact parathyroid hormone decreased from 797 ± 178 pg/mL to 253 ± 25 pg/mL, and the parathyroid gland volume initially increased during the first week, but thereafter, it gradually decreased with weekly PMIT (wPMIT). Finally, the parathyroid gland volume decreased from 1.27 ± 1.06 cm^3 to 0.24 ± 0.15 cm^3 after wPMIT. The benefits of our method were confirmed on weekly ultrasonographic examinations, which detailed the gradual reduction in gland size following an initial increase after the first injection. Therefore, we conclude that our carefully implemented PMIT method would be an effective treatment against refractory secondary HPT. **Key Words:** Intact parathyroid hormone, Parathyroid gland, Secondary hyperparathyroidism, Ultrasonographic examination, Weekly percutaneous maxacalcitol injection therapy.

Percutaneous injection therapy has been performed for secondary hyperparathyroidism (SHPT) as opposed medical treatment. Percutaneous ethanol injection therapy (PEIT) is well known for effectively decreasing parathyroid gland tissue, but because of grave side effects, namely necrosis of the circumferential tissues, PEIT has had limited applications to date.

According to guidelines for SHPT in Japan (1), therapy is limited to surgical or ordinary drug treatments. Recently, the percutaneous injection therapy method using vitamin D was applied in the treatment of SHPT (2–7). Although the procedures for vitamin D injection were not standardized, herein we report on three successful cases and summarize recent reports. As calcimimetics, newly introduced agents, may help to control SHPT, the roles of vitamin D (analogs) and surgical or medical parathyroidectomy need to be reevaluated in the calcimimetic era (8). On this basis, we have developed an outpatient treatment regimen using percutaneous maxacalcitol injection therapy (PMIT) on a weekly basis for 4–6 weeks following dialysis. We found this treatment to be effective, as confirmed by changes in the ultrasonographic findings, at least in the early treatment phase.

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Address correspondence and reprint requests to Dr Atsushi Saito, 31-9 Saiwai-cho, Himi City, Toyama 935-8531, Japan. Email: atsu@kanazawa-med.ac.jp

PATIENTS AND METHODS

Three chronic dialysis patients with severe secondary hyperparathyroidism were evaluated at Honma Hospital (Table 1). Their body mass index was 18.0–22.8 with a mean of 20.6. One of the patients was administered oral calcitriol therapy, and two patients were administered maxacalcitol (OCT) intravenously. All of these cases were resistant to vitamin D therapy and had elevated serum bone metabolic markers such as bone alkaline phosphatase (BAP) and osteocalcin (OC). They had been on maintenance dialysis for 12–18 years and underwent dialysis

TABLE 1. *Clinical profiles of the patients*

Case No.	1	2	3
Demographic data			
Gender	Female	Female	Male
Underlying renal disease	CGN	ADPKD	CGN
Age (years)	58	65	65
Duration of hemodialysis (years)	18	12	14
Body mass index	18.0	22.8	21.1
Baseline date			
Serum intact PTH (pg/mL)	670	720	1000
Serum whole PTH (pg/mL)	250	276	300
Serum adjusted calcium (mg/dL)	11.4	10.5	10.3
Serum phosphorus (mg/dL)	7.8	5.8	6.7
Serum BAP (U/L)	22	33	40
Serum osteocalcin (ng/mL)	280	180	190
Number of enlarged PTGs	2	1	1
Total volume of enlarged PTGs (cm^3)	2.65/0.38	0.50	1.54

ADPKD, autosomal dominant polycystic kidney disease; BAP, bone alkaline phosphatase; CGN, chronic glomerulonephritis; PTGs, parathyroid glands; PTH, parathyroid hormone.

for 240 min, three times per week with polysulfone membrane dialyzers. The dialysate calcium concentration was 1.5 mmol/L in all patients. The Kt/V was 1.36–1.70 with a mean of 1.50. The blood flow rate was 200 mL/min, and the dialysate flow rate was 500 mL/min. Heparin calcium was used as an anticoagulation agent in hemodialysis. Heparin calcium was initially administered at a dose of 1000 U by bolus injection, then followed by 500 U/hr. We intended to control the activated clotting time (ACT) to approximately 170–240 s. If they were administered PMIT, an anticoagulation agent was injected near the end of dialysis, within the final one hour.

This study was approved by the local medical ethics committee, and informed consent was obtained from each patient.

Weekly percutaneous maxacalcitol injection therapy

The enlarged parathyroid glands were examined by ultrasonography (SONOS-4500; Agilent Technologies, Santa Clara, CA, USA) while paying attention to hypervascularity demonstrated in the color Doppler mode, as well as the gland size. The size of the enlarged parathyroid glands was estimated by three-dimensional measurement ($\pi/6 \times a \times b \times c$). Furthermore, we obtained Technetium-99m-methoxyisonitrile (MIBI) scintigraphy of the abnormal parathyroid glands. We confirmed parathyroid cells at aspiration biopsy in the right parathyroid gland of patient 1.

PMIT was performed under ultrasonographic guidance (SSA-250A; Toshiba, Tokyo, Japan) with a puncture support scanner (SMA-736SA: 7.5 MHz; Toshiba), using the same type of needle developed for PEIT (PEIT needle 22G × 150 mm; Hakko, Nagano, Japan). The needle and its extension tubing were primed with OCT, approximately 0.7 mL, in preparation for the injection of OCT solution. We provided a local anesthesia for all patients with 1% lidocaine, which was injected approximately 3 mL away from the puncture site. Under ultrasonographic guidance, the needle was inserted into the center of the parathyroid gland. If the parathyroid gland was large, we injected various sites.

wPMIT was performed once a week following the same method for 4–6 weeks. The injected volume of OCT solution was almost 80% of each gland (mean dose of OCT, 4.60 ± 3.50 μg) and the concentration of the OCT solution was 5 μg/mL (Table 2) (2–7).

Laboratory measurements

The levels of serum intact parathyroid hormone (iPTH), whole PTH (wPTH), BAP, OC, adjusted calcium (adjusted Ca [mg/dL] = Ca [mg/dL] + 4-albumin [g/dl]) (9), and inorganic phosphorus, and other biochemical data were obtained before starting wPMIT and then weekly during the administration of wPMIT in all patients.

Statistical analysis

The laboratory data and changes in the parathyroid gland volume and the parathyroid gland volume ratio were analyzed by Student's paired *t*-test. A P value of <0.05 was considered significant.

RESULTS

As shown in Table 1, serum iPTH, wPTH, adjusted Ca, and P levels were increased in all cases. In addition, BAP and OC were also increased in all cases, suggesting refractory SHPT and a high bone turn-

TABLE 2. *Published methods of vitamin D injection therapy*

Author	Volume of injected solution	Concentration of solution (μg/mL)	Mean dose (μg)	Frequency of injection
Calcitriol				
Kitaoka et al. (2)	70–90% of PTG volume	1	1.88 ± 0.35	3 times/week, total 6 times
Kitaoka et al. (3)	ND	1 or 2	ND	3 times/week
Shiizaki et al. (4)	Equal to PTG volume	1	2.33 ± 1.00	Consecutively, total of 5–10 times
Nakanishi et al. (5)	200–300% of PTG volume	1	ND	1 time/1 PTG
Maxacalcitol				
Yamamoto et al. (6)	2 mL	5	10	1 time only
Shiizaki et al. (7)	Equal to PTG volume	5 or 10	15.80 ± 5.25	6 times/week, total 6 times
Our method	80% of PTG volume	5	4.60 ± 3.50	1 time/week, total 4(–6) times

ND, not described; PTG, parathyroid gland.

over. The average volume of the four enlarged parathyroid glands was 1.27 ± 1.06 cm^3 (Table 1).

The iPTH was greatly decreased in two cases from 3 months of wPMIT, but in one case the amount was not as large; however, the iPTH was significantly decreased in all cases after 6 months. The serum iPTH was 253 ± 25 pg/mL after 12 months ($P < 0.05$, Fig. 1). The serum wPTH decreased from 275 ± 25 pg/mL to 116 ± 27 pg/mL ($P < 0.05$, Fig. 1). Similarly, osteocalcin decreased from 217 ± 55 ng/mL to 73 ± 40 ng/mL ($P < 0.01$). BAP, another bone metabolism marker, also decreased from 31.7 ± 9.1 U/L to 15.7 ± 2.5 U/L.

When the PMIT therapy was finished, OCT therapy was administered intravenously. There were no signs of severe hypercalcemia, exceeding an adjusted Ca level of 12 mg/dL or any of the attendant complications such as a disturbance of consciousness. In this study, the adjusted Ca, P, and calcium phosphorus product did not significantly change.

Alteration of parathyroid gland size

We confirmed that the volume of the parathyroid gland gradually changed. The parathyroid gland volume initially increased during the first week; thereafter, it gradually decreased with wPMIT. Twelve months later it had markedly decreased in case 1 (right), case 2 (right), and case 3 (left), although case 1 (left) displayed almost no change (Fig. 2). A similar tendency was seen when a volume change was reviewed with three parathyroid glands in which the volumes were >0.5 cm^3. While all four parathyroid glands enlarged in the first week, by the third week all had decreased significantly with wPMIT (Fig. 3). The parathyroid gland volume decreased from 1.27 ± 1.06 cm^3 to 0.24 ± 0.15 cm^3 after wPMIT ($P < 0.05$).

The volume of the glands was expressed as a volume ratio that was calculated based on the volume prior to the first PMIT as being 100%. The volume enlarged to approximately 120% one week after the initial wPMIT, and it significantly decreased afterwards, such that the volume ratio 12 months later was 19% of the initial volume ($P < 0.001$).

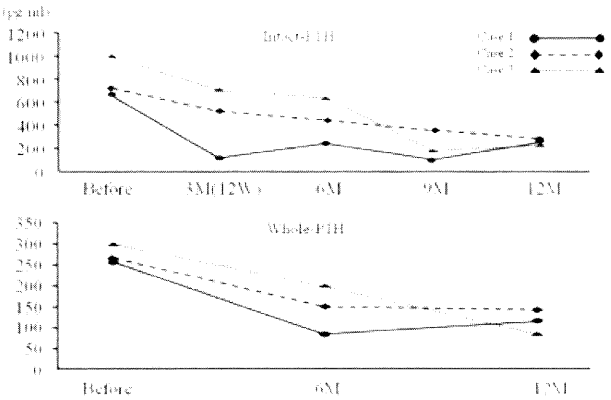

FIG. 1. Alterations of intact- and whole-parathyroid hormone (PTH) levels after maxacalcitol injection therapy.

DISCUSSION

Secondary hyperparathyroidism has been recognized as a risk factor of not only bone metabolism disorders, but also cardiovascular system complications in hemodialysis patients (10). The administration of vitamin D, its analog, or calcimimetic therapy has been performed, as well as dietary controls and prevention of phosphate absorption as the main medical treatments. OCT is one of the effective agents for SHPT (11) without the development of severe hypercalcemia. SHPT has progressed with nodular hyperplasia, and has become resistant to medical therapy (12). In the advanced stage of SHPT, severe hypercalcemia can develop, and intravenous vitamin D therapy or calcimimetic agents as well as surgical treatment are required to control SPTH. Percutaneous therapy directed to the parathyroid gland has been developed in Japan with marked parathyroid retraction in histology (7,13).

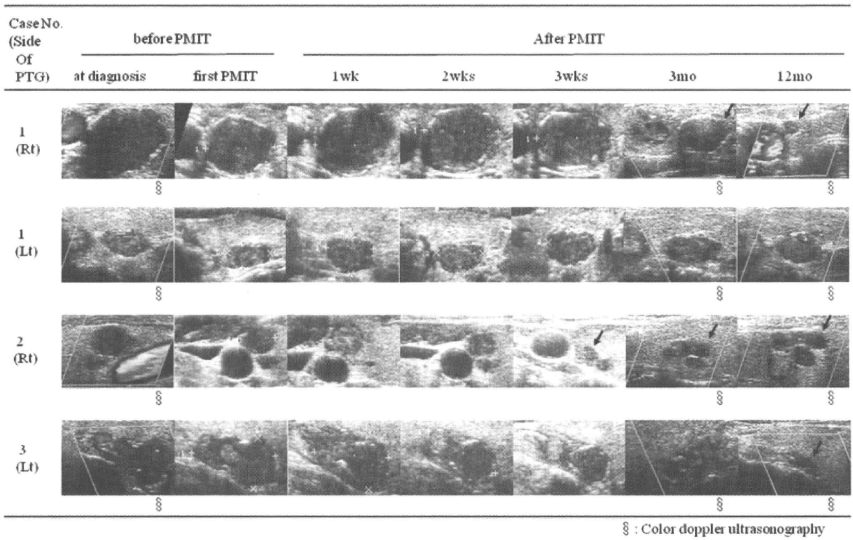

FIG. 2. Ultrasonographic changes of the parathyroid glands with percutaneous maxacalcitol injection therapy (PMIT). The parathyroid gland (PTG) volume initially increased during the first week. Thereafter, the parathyroid gland volume gradually decreased with wPMIT. Twelve months later, it had decreased conspicuously in case 1 (right), case 2 (right), and case 3 (left), but case 1 (left) displayed almost no change.

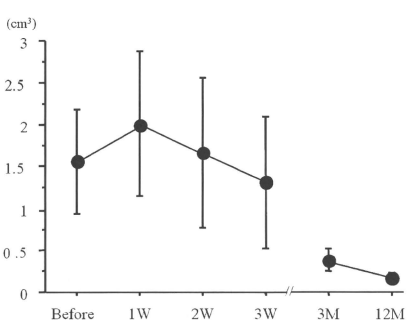

FIG. 3. Changes in the parathyroid gland size (>0.5 cm^3). A similar tendency was seen when a volume change was reviewed with three parathyroid glands in which the volumes were >0.5 cm^3. While all four parathyroid glands enlarged in the first week, by the third week all had decreased significantly with wPMIT.

Initially, ethanol was used for percutaneous injection therapy that was performed for SHPT patients with nodular hyperplasia. It has been recognized as a specific therapy administered by skilled specialists because it can cause adverse complications such as recurrent nerve paralysis or adhesions to the surrounding tissues.

Otherwise, a decrease in the activity of the vitamin D receptor (VDR) and a reduction of Ca sensitivity occurred in the parathyroid gland that had developed nodular hyperplasia, and it became refractory to medical treatment. Therefore, an injection method using the direct injection of a high concentration of vitamin D into the parathyroid gland was tried. In addition, PMIT was designed as a treatment without adverse side effects such as recurrent nerve paralysis caused by adhesion formation. As for the effect of PMIT, Shiizaki et al. (7) reported apoptosis of parathyroid cells by PMIT using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method. PMIT decreased the volume of parathyroid tissue with favorable clinical effects. In addition,

they also reported (13) that a combination of direct OCT injection and intravenous OCT administration induced more expression of Ca-sensing receptor and vitamin D receptor on parathyroid cells. Then they confirmed the improvement of skeletal changes in SHPT by histopathological and histomorphometrical analyses.

We confirmed the transient increase of the parathyroid gland size early after injection. There are some descriptions about enlargement of the parathyroid glands; for example, Kitaoka et al. (3) reported parathyroid gland expansion, possibly by inflammation, two weeks after vitamin D injection therapy in 1995. In 2003 they also found that the parathyroid glands slightly increased just after three times per week of vitamin D injections, and decreased gradually after 2–6 weeks (2). Furthermore, Yamamoto et al. (6) reported the histological changes of enlarged parathyroid glands as inflammatory changes with hemorrhagic necrosis and adhesions to the surrounding tissue at a week after PMIT. Thus it was thought that hemorrhagic necrosis in PTG was a favorable result of PMIT. It seems that changes occur in enlarged parathyroid glands causing degeneration of the parathyroid cells and apoptosis; however, adhesions were considered to indicate an overdose of injected OCT solution.

In all our patients, iPTH was remarkably decreased, but the alteration rate of iPTH after PMIT was different in each patient. The relationship between the explanation of parathyroid glands after PMIT and the alteration rate of iPTH is not sufficiently clear, however. An hypothesis exists suggesting that iPTH may improve rapidly in diffuse hyperplasia, but only slowly with nodular hyperplasia.

On this point, an inadequate volume of injected agents, compared to that in PEIT, has been noted in the reported percutaneous vitamin D injection therapy trials (Table 2), because PEIT has strict guidelines and the injection volume is ≤80%, compared to the volume of the enlarged parathyroid gland at each injection (1). It is reported that the PMIT methods administered a volume overdose of the injected agents, because the effects are dose dependent.

Thus, it is considered that our method largely followed the PEIT guidelines, as well as decreased the parathyroid glands and iPTH as noted in previous studies. In addition, it is possible that our method greatly reduced the total volume of the agents used. We considered that our method did not cause severe complications, such as surrounding adhesions; however, this assumption is still not clear because our patients did not undergo parathyroidectomy. In addition, it is considered that our method measuring parathyroid gland volume every week was effective as an indicator of the efficacy in the early phase.

We now control refractory SHPT according to the Japanese Society for Dialysis Therapy (JSDT) guidelines because of the improved goal of a dialysis patient's prognosis (14). Therefore, we expect that our careful wPMIT method should become a powerful tool to achieve the control of SHPT, even in the new calcimimetic era.

CONCLUSION

In slim patients, we performed PMIT for SHPT from 4–6 times after dialysis once a week, and confirmed the efficacy on ultrasonography. We expected that this method may be helpful in controlling refractory SHPT, even after treatment of calcimimetic agents.

Disclosure: All authors certify that there are no conflict of interests related to this article.

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Therapeutic Apheresis and Dialysis

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Long-Term Natural History of Acquired Cystic Disease of the Kidney

Isao Ishikawa,[1] Satoshi Hayama,[1] Kyoko Morita,[1] Tetsuya Nakazawa,[1] Hitoshi Yokoyama,[2] Ryumon Honda,[2] Kyoko Satoh,[3] and Tatsuyuki Kakuma[3]

[1]Department of Internal Medicine, Division of Nephrology, Asanogawa General Hospital, and [2]Kanazawa Medical University, Kanazawa, and [3]Biostatistics Center, Kurume University, Kurume, Japan

Abstract: Patients with acquired cystic disease of the kidney (ACDK) were followed longitudinally over an average of 21.7 ± 5.4 years to determine the natural history of the disease; that is, how big the kidneys become, when the kidney size reaches a plateau, and when the size regresses. Twenty-seven male and 20 female patients with chronic glomerulonephritis treated at our hospital were investigated. CT scans were performed once a year and kidney volume was measured. Two different quadratic curves with a node of 5.2 years for males and 2.5 years for females after the start of hemodialysis were fitted to log-transformed kidney volume to the duration of hemodialysis using a linear mixed model. The maximum kidney volume in male patients was obtained 21.1 years after the start of hemodialysis using this model. Peak values of kidney volume were demonstrated in 19 of 26 cases during the observation period. The median peak value (interquartile range) of bilateral kidney volumes was 274 (165–849) mL/1.73 m^2 occurring 19.1 ± 4.5 years after the start of dialysis. In one male patient who had undergone nephrectomy due to renal cell carcinoma and in two of the remaining 26 male patients, the maximum kidney volume of 782 (residual kidney), 1151, and 1129 mL regressed to 428, 616, and 847 mL (reduction rate: 45.3, 46.5, and 25.0%) at 20.6, 25.4, and 23.1 years after the start of hemodialysis, respectively. Kidney enlargement due to ACDK reached a plateau after 21.1 years of hemodialysis in the male patients. Partial regression of severe ACDK may occur naturally after long-term hemodialysis without renal transplantation. **Key Words:** Acquired cystic disease, Kidney, Computed tomography scan, Kidney volume, Long-term hemodialysis, Spontaneous regression.

Since renal cell carcinoma (RCC) frequently develops in dialysis patients with acquired cystic disease of the kidney (ACDK) (1–3), our dialysis center performs periodic screening using ultrasound examination or CT scan for hemodialysis patients at high risk of developing RCC. After the start of dialysis diseased kidneys become smaller because of renal parenchymal atrophy (nephron loss) (4) and thereafter become larger due to the development of acquired renal cysts, depending on the duration of hemodialysis (4). Enlarged kidneys due to acquired renal cysts carry a high risk of developing RCC (1,2); moreover, a gender difference is observed in ACDK and the risk of developing acquired renal cysts, with the incidence of RCC being higher in males than females (1,5). However, the long-term natural history of ACDK is not known; that is, how big the kidneys become, and when the kidneys reach a plateau or regress; therefore, long-term follow-up of diseased kidneys in patients with chronic glomerulonephritis was performed in this study.

PATIENTS AND METHODS

One hundred and seventy-eight patients underwent periodic renal CT examination between September 2006 and August 2007 in our dialysis unit. The original disease in 90 of these patients was the diagnosis or suspicion of chronic glomerulonephritis. Of these 90 patients, 34 male and 27 female had received hemodialysis for more than 10 years and the kidney volumes were measured in 31 male and 24 female patients who received a CT scan every year from the start of dialysis or from the third year after starting

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Address correspondence and reprint requests to Dr Isao Ishikawa, Division of Nephrology, Department of Internal Medicine, Asanogawa General Hospital, 83 KosakaNaka, Kanazawa 920-8621. Japan. Email: isikawai@kanazawa-med.ac.jp

dialysis. Finally, kidney volumes were measured in 27 male and 20 female patients, excluding patients who eventually received renal transplantation. These patients were observed until August 2008. Two male and one female patients were included in our previous 20-year follow-up study (6). The mean duration of hemodialysis (±SD) in 47 patients was 21.7 ± 5.4 years (males: 21.5 ± 4.9, $n = 27$; females: 22.0 ± 6.2, $n = 20$). The study protocol was approved by the Ethics Committee of Kanazawa Medical University and informed consent (oral or written) was obtained from each patient.

Kidney volume was measured by the conventional method (4,7); that is, as a summation from the upper to lower pole of the slice area multiplied by slice thickness using plain CT scan once a year over 10–32 years from the start of dialysis. Measurement of the renal area of the CT slices was made using DICOM data and POP-Net essential software (version 4.1c; Image One, Tokyo, Japan). The kidney volume was described as the summation of the bilateral kidney volumes. If the kidney volume fluctuated within ±25% of the kidney volume of the previous year's measurement, it was considered to have reached a plateau. The kidney volume was corrected by the standard body surface area (BSA) of 1.73 m^2, and the coefficient variation of kidney volume measurement in our method was 4.6% (8).

The kidney size in male patients on long-term hemodialysis varied remarkably from small to large according to the severity of acquired renal cysts, and the number of patients on long-term hemodialysis in each year was the highest at 15 and 16 years after the start of hemodialysis. Therefore, a variety of biochemical indicators and clinical parameters were analyzed in 23 patients 15 years after the start of hemodialysis, comparing patients with smaller and larger kidney volumes. The parameters examined were: Kt/V, blood urea nitrogen, serum creatinine (measured values by the Jaffe method in the past were corrected to the value by the recent enzymatic method), and β_2 microglobulin for dialysis efficiency; total cholesterol, serum albumin, and BMI for nutritional state; and mean blood pressure, hemoglobin, parathyroid hormone (PTH: the measured values by highly sensitive PTH in the past were corrected to the value by the recent intact parathyroid hormone [iPTH] values), alkaline phosphatase (ALP), plasma renin activity (PRA), C-reactive protein (CRP) for inflammatory reaction, and testosterone levels.

The distribution of each variable was graphically inspected, and kidney volumes, levels of PTH, ALP, PRA, and CRP were judged non-normally distributed. The median and interquartile range (IQR) (25–75% percentiles) were reported as summary statistics for these variables instead of the arithmetic mean ± SD for normally distributed data. In addition, non-normally distributed variables were log-transformed before statistical analyses if necessary.

The annual data of kidney volumes after the start of hemodialysis unfortunately had a few missing values. These missing values occurred at any duration of hemodialysis, but the amount of missing data increased with increasing hemodialysis duration. To this end, data more than 26 years after the start of hemodialysis were excluded from statistical analysis. One male patient who had undergone unilateral nephrectomy due to RCC was excluded from statistical analysis because the kidneys could no longer be measured bilaterally. Moreover, the analysis was made using the log-transformed value over the kidney volume.

A linear mixed model (9) was employed to model the time course of kidney volume after the start of hemodialysis. Although a cubic curve may be used to approximate the mean time profile of kidney volume, it would make the interpretation of model parameters difficult in clinical terms. In order to avoid the difficulties, the mean time course was modeled with two spline quadratic curves with a node (10). One quadratic curve represents the downward trend while other captures the upward trend; the turning point or intersection of the two quadratic curves is the node.

Denoting Y_{it} as the log-transformed kidney volume for patient i ($i = 1, \ldots, 27$), and at the time t measured (in years) after the start of hemodialysis ($t = 0, 1, \ldots, 25$), repeated measurements of the kidney volumes were modeled as:

$$Y_{it} = \beta_0 + \beta_1(t - t^*)_- + \beta_2\left((t - t^*)_-\right)^2 + \beta_3(t - t^*)_+ + \beta_4\left((t - t^*)_+\right)^2 + b_i + \varepsilon_{it}$$

where $(t-t^*)_+$ and $(t-t^*)_-$ are spline functions with node t^* defined, respectively, as:

$$(t - t^*)_+ = \max(0(t - t^*)), \quad (t - t^*)_- = (t - t^*) \pm (t - t^*).$$

β_0, β_1, β_2, β_3, and β_4 are regression parameters, b_i is the random intercept for each patient and independent from random error ε_{it}.

Graphical inspection on the mean profile of kidney volume suggested that the node location should be approximately 4–6 years after the start of hemodialysis in male patients. In order to determine the best node location, every 0.1 years between 4 and 6 years after the start of hemodialysis was used as a candidate node, and the Akaike's Information Criterion (AIC) of the model with different nodes was com-

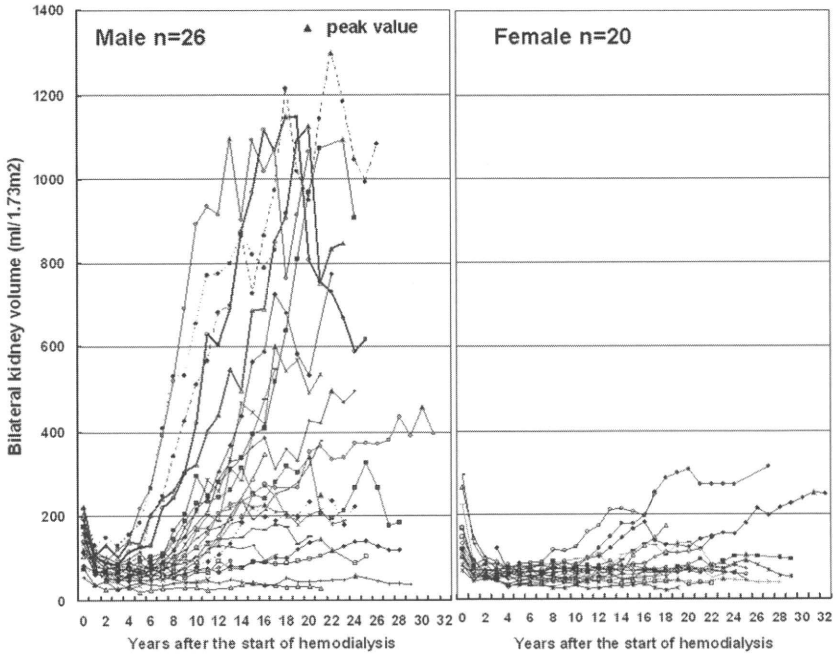


FIG. 1. Longitudinal changes in kidney volumes in 26 males and 20 females on long-term (>10 years) hemodialysis due to chronic glomerulonephritis (peak values represented by triangles).

pared. The AIC reached the minimum when the node was 5.2; therefore, the node value 5.2 was chosen as the optimal node. The same approach was applied to female patients and a node value 2.5 was chosen as the optimal node.

The Bonferroni test was used to control Type I error when multiple comparisons between the maximum kidney volume and kidney volumes after the maximum value were performed. The linear mixed model with spline function was fitted using the SAS mixed procedure (SAS Institute, Cary, NC, USA).

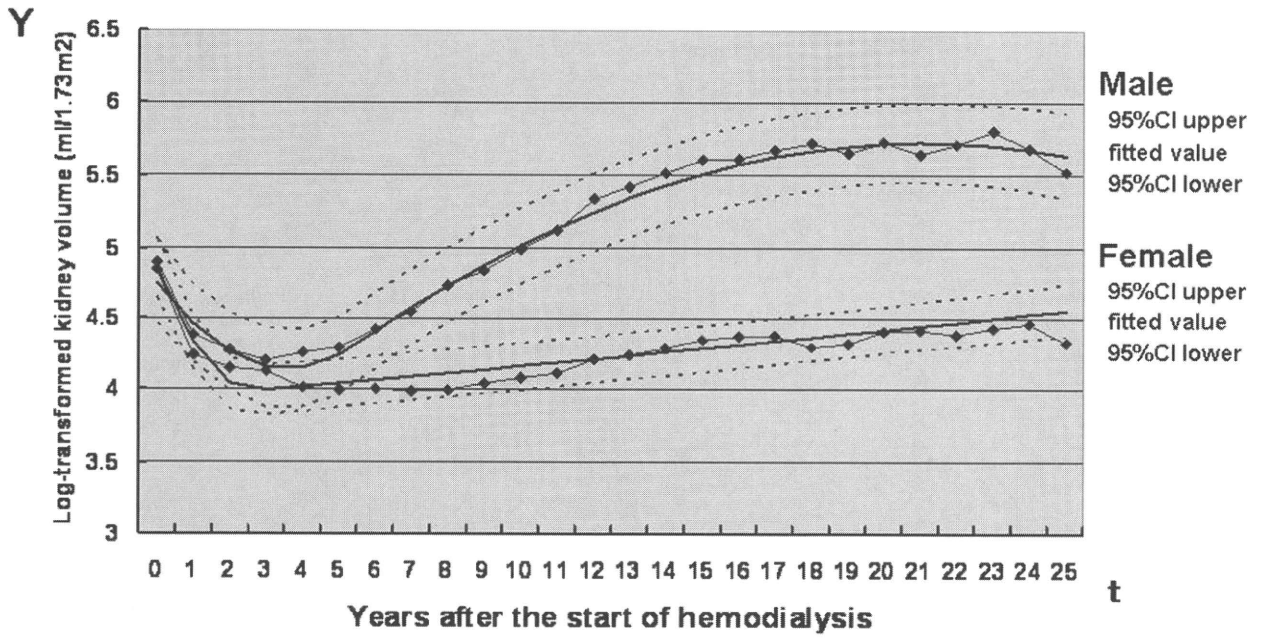
Student's non-paired *t*-test or the Mann–Whitney *U*-test was used to analyze differences between two groups. A *P*-value <0.05 was considered significant. Statistical analyses were performed using StatView version 5.0 (SAS Institute, Cary, NC, USA).

RESULTS

The relation of kidney volume to the duration of hemodialysis in each patient is shown in Figure 1. In male patients the peak kidney volumes occurred in 19 of 26 cases during the observation period. The median peak kidney volume was 274 (IQR 165–849) mL/1.73 m^2, and the mean interval to peak value was 19.1 ± 4.5 years after the start of dialysis. The median kidney volume was 71 (IQR 55–85) mL/1.73 m^2 $n = 24$, 320 (186–723) mL/1.73 m^2 $n = 19$, 280 (157–696) mL/1.73 m^2 $n = 19$, and 347 (164–740) mL/1.73 m^2 $n = 18$, after 3, 18, 19, and 20 years of hemodialysis, respectively. In female patients the peak kidney volumes were shown in 12 of 20 cases during the observation period. The median peak kidney volume was 80 (IQR 65–95) mL/1.73 m^2, and the mean interval to peak value was 21.6 ± 4.6 years after the start of dialysis. The median kidney volume was 62 (IQR 49–71) mL/1.73 m^2 $n = 20$, 66 (54–107) mL/1.73 m^2 $n = 15$, 65 (62–102) mL/1.73 m^2 $n = 14$, and 76 (68–112) mL/1.73 m^2 $n = 13$, after 3, 18, 19, and 20 years of hemodialysis. The log-transformed kidney values from 5 to 25 years after the start of hemodialysis were significantly greater in male patients than in female patients.

Fitted log-transformed kidney volumes with 95% confidence intervals (CI) were obtained from the estimated model parameters. Figure 2 shows the fitted value, 95% CI, and observed mean with the estimated regression parameters. Since the mean of the log-transformed measured value and estimated mean by the model are quite similar, this model represented the kidney volume changes to the year after the start of hemodialysis in male patients quite well. Based on the model, the time that the minimum (maximum) kidney volume is attained was calculated to be 3.6 years (21.1 years) after the start of hemodialysis. The estimated maximum kidney volume was not significantly different from the mean kidney volume at 22, 23, 24, or 25 years after the start of hemodialysis (Fig. 2).

In female patients, curve fitting showed that the insignificant positive β4 indicated a slow linear



Male	Solution of fixed effects				
parameter	estimated value	SE	DF	t-value	Pr>|t|
β 0	4.2680	0.1404	25	30.40	<.0001
β 1	-0.1545	0.0503	511	-3.07	0.0023
β 2	0.0478	0.0102	511	4.68	<.0001
β 3	0.1826	0.0113	511	16.18	<.0001
β 4	-0.0057	0.0006	511	-9.48	<.0001

Female	Solution of fixed effects				
parameter	estimated value	SE	DF	t-value	Pr>|t|
β 0	3.9879	0.0894	19	44.61	<.0001
β 1	0.0509	0.1091	397	0.47	0.6412
β 2	0.1194	0.0449	397	2.66	0.0081
β 3	0.0220	0.0083	397	2.64	0.0085
β 4	0.0002	0.0004	397	0.40	0.6905

FIG. 2. Relationship between the log-transformed kidney volume (Y) and duration of hemodialysis (t): the fitted value (solid line), 95% CI (dashed line), and observed mean (solid line with diamonds) with the estimated regression parameters. Model equation is $Y_{it} = \beta_0 + \beta_1(t - t^*)_- + \beta_2((t - t^*)_-)^2 + \beta_3(t - t^*)_+ + \beta_4((t - t^*)_+)^2 + b_i + \varepsilon_{it}$.

increase in kidney volume during long-term hemodialysis instead of a second quadratic curve with a downward trend, which differed from that in male patients. The minimum kidney volume in female patients was calculated to occur at 2.5 years and the maximum kidney volume was estimated to occur more than 26 years after the start of hemodialysis.

On comparison of the relationship between kidney volume and various biochemical and clinical parameters in 23 male patients 15 years after the start of hemodialysis, there were no significant differences in these parameters between the male patients with smaller ($n = 12$) and larger ($n = 11$) kidney volumes, except for serum creatinine levels (Table 1).

Spontaneous partial regression (more than 25% of the peak value of kidney volume) of ACDK was observed despite the continuation of hemodialysis treatment in one male patient with uninephrectomy and two of 26 male patients. Kidney volumes in these three patients were 159, 193, and 223 mL/1.73 m^2 at the start of dialysis, respectively. Two, three, and three years after the start of dialysis, the kidney volumes showed minimum (trough) values of 150, 87, and 103 mL/1.73 m^2, and thereafter the kidney volumes enlarged rapidly, respectively; and at 17, 18, and 20 years after the start of dialysis these patients reached maximum (peak) values of 782 (left kidney volume), 1151, and 1129 mL/1.73 m^2 (Fig. 2). At 20.6 years (3 years after the peak), 25.4 years (7 years after the peak), and 23.1 years (3 years after the peak) after the start of hemodialysis, the kidney volume decreased to 428 (reduction rate: 45.3%), 616 (reduction rate 46.5%), and 847 mL/1.73 m^2 (reduction rate 25.0%), respectively. Spontaneous regression in female patients was seen in seven patients; however, the peak values of kidney volume were below 132 mL/1.73 m^2 in these seven patients.

TABLE 1. *Comparison of clinical parameters in male patients with smaller and larger kidney volumes 15 years after the start of hemodialysis*

	With smaller kidney volume (40–254 mL)	With larger kidney volume (290–1095 mL)	*P*
n	12	11	
Age (years)	54.3 ± 9.7	54.7 ± 7.3	NS
Mean BP (mmHg)	106 ± 7	101 ± 12	NS
BMI	20.6 ± 2.4	22.7 ± 2.7	NS
Hb (g/dL)	10.1 ± 1.2	11.1 ± 1.3	NS
BUN (mg/dL)	71 ± 10	73 ± 20	NS
Serum creatinine (mg/dL)	13.7 ± 1.4	15.9 ± 2.2	$P < 0.01$
Kt/V	1.50 ± 0.23	1.43 ± 0.15	NS
Serum albumin (g/dL)	4.2 ± 0.4	4.0 ± 0.2	NS
Total cholesterol (mg/dL)	175 ± 41	171 ± 33	NS
β_2 Microglobulin (μg/dL)	29.1 ± 4.8	28.8 ± 3.4	NS
iPTH (pg/mL; median [IQR])	187 (128–346)	187 (84–639)	NS
Alkaline phosphatase (U/L)	136 (105–293)	158 (134–199)	NS
CRP (mg/dL; median [IQR]) (*n*)	0.18 (0.09–0.28) (7)	0.08 (0.08–0.13)(10)	NS
PRA (ng/mL/h; median [IQR])	1.65 (0.65–6.80)	2.70 (0.55–4.18)	NS
Testosterone (ng/mL)	5.07 ± 1.46	5.36 ± 1.74	NS

BUN, blood urea nitrogen; CRP, C-reactive protein; iPTH, intact parathyroid hormone; IQR, interquartile range; PRA, plasma renin activity.

Case presentations showing spontaneous partial regression of severe ACDK

Patient 1 was a 41-year-old (at the start of hemodialysis) male; a right nephrectomy due to RCC was performed shortly after the introduction of hemodialysis (2.2 years after starting hemodialysis) (Fig. 3). Calcification of the left renal artery was prominent on CT image. Renal cysts in the left kidney became smaller 20.6 years after the start of hemodialysis, compared with those 15.2 or 17.3 years after the start of hemodialysis. The patient's general condition remained unremarkable during this period.

Patient 2 was a 41-year-old (at the start of hemodialysis) male who demonstrated the minimum kidney volume on CT imaging 3.3 years after the start of hemodialysis (Fig. 4). Thereafter, the diseased kidney began to enlarge because of the development of acquired renal cysts. The individual renal cyst size 25.4 years after the start of hemodialysis was decreased compared with that 13.9 or 18.4 years after the start of hemodialysis at which time the patient demonstrated large acquired renal cysts in the bilateral kidneys. A cyst with calcified cyst wall in the right kidney developed between 13.9 and 18.4 years after the start of hemodialysis, and the size of this cyst remained the same. The patient was not malnourished and showed a stable condition during this period. The patient had complained of gross hematuria since February 2008 and RCC was suspected in ACDK without doing a dynamic CT scan because of iodine allergy. The patient was followed by plain MRI.

Regarding the development of RCC in these male patients, nephrectomy was carried out in one case immediately after the start of hemodialysis as previously stated, and another nephrectomy due to RCC was performed in 2008; one further nephrectomy in early 2010. In addition, three additional patients are suspected of developing RCC in ACDK by periodic CT screening and are currently being followed because there is almost no enhancement on dynamic CT scans.

DISCUSSION

Follow-up studies of ACDK have been reported by Ishikawa et al. (6,8,11) and Levine et al. (12); however, the duration of hemodialysis was not very long-term and the numbers of patients were insufficient (8,11,12). In this study, we followed patients long-term with a mean of 21.7 ± 5.4 years after the start of hemodialysis in 27 male and 20 female patients. The natural history of ACDK was partially clarified in this study. When male patients with end-stage kidney disease due to chronic glomerulonephritis were introduced to hemodialysis, the kidney volume was minimized because of the loss of hypertrophied nephrons 3.6 years after the start of hemodialysis, and thereafter, the kidney enlarged due to the development of acquired renal cysts (4). The maximum kidney volume was obtained at 21.1 years after the start of hemodialysis and thereafter the kidney volume showed a plateau. It also became clear that in a subset of the patients, the kidney volume partially regressed after the peak volume was reached. Furthermore, even among male patients, there were some patients who showed only minimal

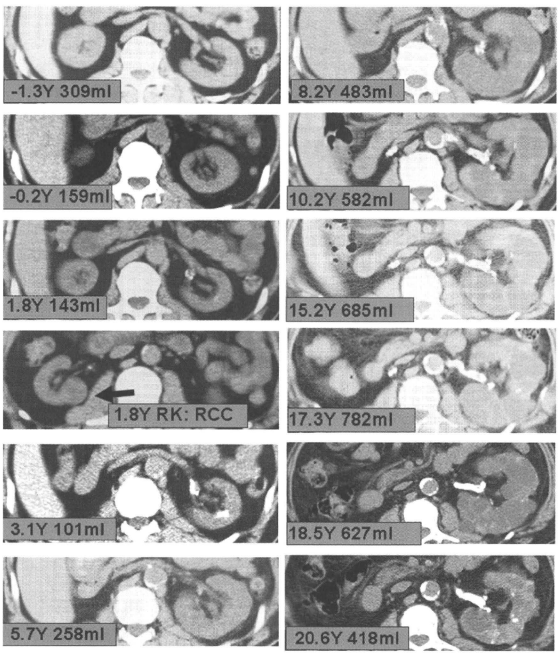


FIG. 3. Moderate degree of regression (spontaneous partial regression) of acquired cystic disease of the kidney (ACDK) during long-term hemodialysis without renal transplantation (41-year-old male): left enlarged kidney 15.2 years and 17.3 years after the start of hemodialysis showed regression 20.6 years after the start of hemodialysis.

enlargement of kidney volume due to the development of several acquired renal cysts, as in female patients.

Among male patients with smaller and larger kidney volumes 15 years after the start of hemodialysis, there were no differences in mean age, BMI, hemoglobin, nutrition, dialysis efficacy, parathyroid hormone or CRP, although serum creatinine levels did differ significantly. While the effect of testosterone on cyst development in experimental cystic disease can be considered due to the male predominance (13), the relation of testosterone with kidney volume enlargement in acquired renal cysts was examined in this study with negative results. Furthermore, there was no relationship between kidney volume in ACDK and the renin–aldosterone system.

Moderate-degree or partial regression of ACDK was an unexpected occurrence. Previously regression of ACDK had been shown after successful renal transplantation (14). The regression of ACDK after successful renal transplantation occurs in almost all graft recipients and almost completely (14), the regression of ACDK in long-term hemodialysis patients shows a low incidence and occurs only partially or to a moderate degree. The moderate degree or partial regression of ACDK during long-term hemodialysis in two of three patients was around 40% of the maximum kidney volume and the reduction rate of the kidney volume was similar to that of kidney volume by renal transcatheter arterial embolization for autosomal dominant polycystic disease (ADPKD) (15).

For two of the three patients with ACDK that regressed moderately during long-term hemodialysis, the etiology of this regression may be due to unknown changes (for example, apoptosis of cyst epithelium, reduction of fluid secretion, increase in fluid resorption, and so on) in the mechanism of the development and maintenance of acquired renal cysts, not due to renal artery obstruction, since the regression of ACDK in two patients occurred in the same fashion in both kidneys, excluding the patient with a