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A case–control study of calciphylaxis in Japanese end-stage renal disease patients

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Abstract

Background. Calciphylaxis, also called calcific uremic arteriolopathy, is a rare and often fatal complication of end-stage renal disease and is characterized by painful skin ulceration, necrosis, medial calcification and intimal proliferation of small arteries. Studies in western countries have reported incidences ranging from 1 to 4% in chronic hemodialysis patients. Since no systematic studies of calciphylaxis have ever been performed in Japan, we conducted a nationwide survey and a case–control study to identify the characteristics of calciphylaxis in the Japanese dialysis population.

Methods. Firstly, we sent a questionnaire to 3760 hemodialysis centers in Japan, asking whether calciphylaxis cases had been encountered in the past, and detailed clinical data regarding each case were then collected from the centers. In addition, two control dialysis patients matched for age and duration of hemodialysis to each calciphylaxis case were identified at the participating centers, and their data were analyzed to identify risk factors for calciphylaxis.

Results. Responses to the questionnaire were obtained from 1838 centers (48.3%), and 151 centers reported that a total of 249 cases had been encountered. Sixty-four centers agreed to participate in the case–control study, and detailed clinical data in regard to 67 cases were obtained. In 28 of the 67 cases, a definite diagnosis of calciphylaxis was made by our study group based on the clinical characteristics and skin biopsy findings. A univariate logistic regression model comparing them with 56-matched controls identified warfarin therapy [odds ratio (OR) 11.4, 95% confidence interval (CI)] 2.7–48.1, P = 0.0009], each 1 g/dL decline in serum albumin level (OR 19.8, 95% CI 4.4–89.5, P = 0.0001), each 100 mg/dL increment in plasma glucose level (OR 3.74, 95% CI 1.08–12.9, P = 0.037) and each 1 mg/dL increment in adjusted serum calcium level (OR 3.2, 95% CI 1.63–6.30, P = 0.0008) at the time of diagnosis as significantly associated with calciphylaxis, but no significant associations were found with female gender, vitamin D analog therapy, serum phosphate level, adjusted calcium–phosphate products or serum alkaline–phosphatase level. Warfarin therapy and lower serum albumin levels were still significant risk factors after a multivariate logistic regression model analysis.

Conclusion. The results of this study showed that warfarin therapy and lower serum albumin levels are significant and strong risk factors for the development of calciphylaxis in chronic hemodialysis patients in Japan.

Keywords: albumin; calciphylaxis; hemodialysis; warfarin

Introduction

Calciphylaxis, also called calcific uremic arteriolopathy, is a rare and often fatal complication of end-stage renal disease [1–3]. Calciphylaxis is clinically characterized by painful skin ulceration and necrosis. Pathological examination of biopsied skin shows medial calcification and intimal proliferation of small arteries. Selye *et al.* [4] were the first to use the term 'calciphylaxis', applying it to a condition they observed in rodents, and defining it as 'a condition of hypersensitivity in which, during a critical period after sensitization by a systemic calcifying factor, treatment with certain challengers causes an acute, local calcification followed by inflammation and sclerosis'. A few years after their report, a syndrome of peripheral ischemic tissue necrosis and cutaneous ulceration was reported in uremic patients [5], and because of its resemblance to the skin lesion of the rodent model described by Selye, it was termed calciphylaxis. The characteristic histological features of calciphylaxis in humans are medial calcification and intimal proliferation in small arteries, and although they are clearly different from the characteristic histological features in the rodent model, the term calciphylaxis is being still used [3]. The term calcific uremic arteriolopathy may be more appropriate [2], but calciphylaxis has often been reported in patients who were not uremic.

Previous studies in western countries have reported incidences of calciphylaxis ranging from 1 to 4% in chronic hemodialysis patients [2]. Several risk factors have been suggested, including female gender, obesity, diabetes mellitus, elevated serum phosphate levels, hypercalcemia, hyperparathyroidism, low serum albumin levels and warfarin therapy. Sporadic cases of calciphylaxis have also been reported in Japan, and although its prevalence seems to be very low, no systematic surveys have ever been conducted. We conducted the present study to identify the characteristics of Japanese calciphylaxis patients and to identify risk factors for calciphylaxis in Japanese hemodialysis patients.

Materials and methods

Study population

Nationwide survey and cases. Firstly, we conducted a nationwide survey to recruit calciphylaxis patients by mailing a questionnaire containing the following questions to all 3760 hemodialysis centers that are institutional members of the Japanese Society of Dialysis and Transplantation:

Q1. Has a patient with calciphylaxis ever been encountered at your center?

If answer is 'no', go to Q5.

Q2. What was the total number of calciphylaxis patients encountered at your center? What was the gender of the patients?

Q3. Please state the number of the calciphylaxis patients in each of the following age ranges.

Under 20, 21–30, 31–40, 41–50, 51–60, 61–70, >71.

Q4. Does your center wish to participate in our case–control study?

Q5. Do you know about calciphylaxis (please circle one of the following answers)?

Yes, very well; yes, well; yes, but only the name of the disease; no.

Responses to the questionnaire were received from 1838 (48.3%) of the centers and 64 of them agreed to participate in the case–control study. Clinical data regarding each of the calciphylaxis cases were collected by the study group.

Controls. The controls were selected from the patients undergoing hemodialysis at Keio University Hospital, Saitama Social Insurance Hospital, Yoyogi Station Clinic, Yoshizawa Clinic, Tokorozawa Jin Clinic and Inagi Municipal Hospital. Two controls matched for age (within ±2 years) and duration of hemodialysis (within ±20%) were selected for each case. The control patients were alive at the time of the survey.

Data collection

The clinical records of all cases and controls were reviewed retrospectively, and the following demographic and medical data were abstracted from them: age, gender, primary cause of the end-stage renal disease, total period of hemodialysis and whether the subject was being treated with warfarin, vitamin D or a vitamin D analog currently or at the time of diagnosis of calciphylaxis. A summary of the clinical course of each subject (sites affected, histological features, management of the skin lesions and main therapy) was recorded. Whenever histological slides of skin biopsy specimens were available, they were sent to the study group to be scanned and recorded with a Virtual Slide System (Olympus Co., Ltd, Tokyo, Japan). The slides were used to make a diagnosis by pathologists in our study group. The final diagnosis of calciphylaxis by the core members of the study group was based on the presence of painful purpura, characteristic skin lesions and typical pathological findings in the biopsy specimen, i.e. medial calcification and intimal hyperplasia of small arteries.

The following data, which were recorded at the time of the diagnosis of calciphylaxis at each hemodialysis center, were collected: serum albumin level, serum calcium level, serum phosphate level, serum intact parathyroid hormone level, serum alkaline phosphatase level, total cholesterol concentration and plasma glucose level. The data listed above were also collected for each of the control cases.

Statistical analysis

The following formula was used to calculate the serum adjusted Ca concentration (mg/dL): [measured Ca concentration (mg/dL) + 4.0] – serum albumin concentration (g/dL). Discrete variables at baseline were compared by chi-square tests, and normally distributed continuous variables were compared by Student's *t*-tests.

The risk factors for developing calciphylaxis were identified by performing univariate and multivariate logistic regression analyses. The covariates included in the multivariate models were chosen from the factors identified as significant by using the univariate model. Two-sided P-values <0.05 were considered statistically significant. All data were analyzed using IBM SPSS Statistics 18 software (IBM corporation).

Results

Responses to the questionnaire were obtained from 1838 (48.3%) of the 3760 centers. The responses revealed that a case of calciphylaxis had been encountered at 151 of the centers in the past, and the age and gender distributions of the cases are shown in Table 1. A total of 249 cases had been encountered, and their gender distribution did not differ significantly from that of the total dialysis population in Japan. According to the results of the Wilcoxon rank sum test, the age range of the calciphylaxis patients was significantly lower than the age range of hemodialysis patients in Japan as a whole. Sixty-four of the 151 centers at which a case of calciphylaxis had been encountered agreed to participate in our case–control study, and they sent the clinical data listed above in the 'Materials and methods' section to our study group. The 1687 centers that replied to the questionnaire but at which no cases of calciphylaxis had been encountered answered Q5 (Do you know about calciphylaxis?) as follows: yes, very well, 6.4%; yes, well, 30.3%; yes, but only the name of the disease, 37.7%; no, 28.0%.

These results indicated that only 40% of the physicians who answered the questionnaire at each of the hemodialysis centers knew any more about calciphylaxis than the name of the disease.

Clinical data regarding 67 cases were sent to the study group and analyzed, but 39 of the cases were excluded because of the uncertainty about the diagnosis. The remaining 28 cases were identified as definite cases of calciphylaxis, and their clinical records were used in the case–control study. Data regarding the control cases were then collected from the hemodialysis centers named in the 'Materials and methods' section above. As shown in Table 2, the ages and duration of hemodialysis of the cases and controls were well matched, and the gender distribution of the two groups was not significantly different. The albumin levels were significantly lower, the adjusted calcium concentrations were significantly higher and warfarin therapy was significantly more common in the calciphylaxis cases.

Table 3 shows the results of the univariate logistic analysis. They showed that warfarin therapy [odds ratio (OR)] 11.4, 95% confidence interval (CI) 2.7–48.1, P = 0.0009], each 1 g/dL decline in serum albumin level (OR 19.8, 95% CI 4.4–89.5, P = 0.0001), each 100 mg/dL increment in plasma glucose level (OR 3.74, 95% CI 1.08–12.9, P = 0.037) and each 1 mg/dL increment in adjusted serum calcium level (OR 3.2, 95% CI 1.63–6.30, P = 0.0008) at

Table 1. Age and gender distribution of the controls and cases

Gender ratio and age (years)	All dialysis patients in Japan (as of 31 December 2008) [6]	Calciphylaxis cases
Gender (F/M)	104 252/167 446	89/160
<20	213 (0.08%)	2 (0.8%)
21–30	1421 (0.52%)	4 (1.61%)
31–40	7898 (2.91%)	17 (6.83%)
41–50	20 273 (7.46%)	35 (14.06%)
51–60	52 417 (19.29%)	59 (23.69%)
61–70	81 312 (29.93%)	77 (30.92%)
>71	108 134 (39.80%)	53 (21.29%)

Table 2. Clinical characteristics of the definite calciphylaxis cases and the control patients[a]

	Controls	Cases
Gender	M: 34, F: 22	M: 12, F: 16
Age (years)	58.4 ± 11.1	58.4 ± 10.9
History of dialysis (months)	108.6 ± 85.5	107.5 ± 86.9
Warfarin therapy	4/56 (3/44)	10/22***
Vitamin D therapy	32/56 (25/42)	13/21
Albumin (g/dL)	3.9 ± 0.3	3.2 ± 0.7***
Adjusted Ca (mg/dL)	9.3 ± 0.7	10.0 ± 1.0***
Inorganic phosphate (mg/dL)	5.7 ± 1.2	5.7 ± 2.6
Intact PTH (pg/mL)	246 ± 166	310 ± 358
Alkaline phosphatase (IU/L)	254 ± 133	307 ± 138
Plasma glucose (mg/dL)	118 ± 36	146 ± 60
Total cholesterol (mg/dL)	165 ± 33	163 ± 46
Diabetic nephropathy	18/56	11/28

[a]Values are means ± SD. No record of warfarin therapy was obtained in six cases, and no record of vitamin D analog therapy was obtained in seven cases. Number in parenthesis presents the results of corresponding controls to the cases with medication record.
***$P < 0.001$ in comparison with the controls.

Table 3. Results of using the univariate logistic regression model to identify predictors of calciphylaxis among the parameters measured at the time the diagnosis of calciphylaxis was made[a]

	P-value	OR (95% CI)
Female gender	0.124	2.06 (0.82–5.28)
Warfarin therapy	0.0009	11.4 (2.7–48.1)
Vitamin D therapy	0.960	1.03 (0.39–2.73)
Serum albumin (for each 1 g/dL decline)	0.0001	19.8 (4.4–89.5)
Adjusted Ca (for each 1 mg/dL increment)	0.0008	3.20 (1.63–6.30)
Inorganic phosphate (mg/dL)	0.888	1.02 (0.78–1.33)
Intact PTH (pg/mL)	0.312	1.00 (1.00–1.00)
Alkaline phosphatase (IU/L)	0.117	1.00 (1.00–1.00)
Plasma glucose (for each 100 mg/dL increment)	0.037	3.74 (1.08–12.9)
Total cholesterol (mg/dL)	0.830	1.00 (0.98–1.01)
Diabetic nephropathy	0.517	1.37 (0.53–3.51)

[a]Only 22 cases and their corresponding matched controls were used in the analysis in regard to warfarin therapy. Only 21 cases and their corresponding matched controls were used in the analysis in regard to vitamin D analog therapy.

Table 4. Results of using the multivariate logistic regression model to identify predictors of calciphylaxis at the time the diagnosis of calciphylaxis was made

	P-value	OR (95% CI)
Warfarin therapy	0.013	10.1 (1.63–62.7)
Serum albumin (for each 1 g/dL decline)	0.003	12.7 (2.35–68.6)
Plasma glucose (for each 100 mg/dL increment)	0.309	0.99 (0.97–1.01)

the time of diagnosis were significantly associated with calciphylaxis. On the other hand, there were no significant associations with the previously reported risk factors: female gender, vitamin D analog therapy, serum phosphate level, adjusted calcium–phosphate products or serum alkaline–phosphatase level.

Table 4 shows the adjusted relative risk of calciphylaxis associated with possible risk factors at the time calciphylaxis was diagnosed at each center. Warfarin therapy was still significantly associated, and there was a 10.1-fold higher risk of calciphylaxis. Each 1.0 g/dL decline in the serum albumin level at the time of diagnosis increased the risk of calciphylaxis by 12.7-fold, but the serum glucose level was not a significant risk factor according to the multivariate analysis. Since the calculation of adjusted calcium concentration includes the serum albumin concentration, it was not simultaneously included in the multivariate analysis with the serum albumin concentration. When the adjusted calcium concentration instead of the albumin concentration was used in the multivariate logistic analysis, the adjusted calcium concentration was also found not to be a significant risk factor either.

Discussion

The results of this case–control study identified risk factors for calciphylaxis in Japanese hemodialysis patients. In the univariate logistic analysis, warfarin therapy, lower serum albumin levels, higher adjusted calcium concentrations and higher glucose levels were identified as significant risk factors for developing calciphylaxis. In the multivariate logistic analysis, warfarin therapy and lower serum albumin levels were identified as risk factors, but glucose levels and adjusted calcium concentrations were not.

As stated in the introduction, calciphylaxis was first reported by Selye and defined as a systemic hypersensitivity reaction analogous to the allergic reaction (anaphylaxis) [4]. A case of calcifying panniculitis with renal failure was reported as calciphylaxis in 1968 and that case was accompanied by the presence of large painful lumps in the fat of the thighs and knees, over which the skin rapidly became necrotic. Since the patient also had hyperparathyroidism, the authors speculated that the skin lesions were caused by sensitization to parathyroid hormone (PTH) [5]. Other early case reports of calciphylaxis also speculated that hypersensitivity to PTH played a major role in the development of the skin lesions [7–9]. After these reports, it became evident from the pathological findings that

calciphylaxis in humans is not the same as in the rodent model [1–3]. Furthermore, cases of calciphylaxis without hyperparathyroidism have been reported in both patients with and without renal failure. Since calciphylaxis in humans is now thought to be different from the calciphylaxis in Selye's rodent model, 'calcific uremic arteriolopathy' has been proposed as a more suitable name for the disease. However, since cases of 'calciphylaxis' without uremia have been reported, we chose to use the term calciphylaxis in this paper.

The prevalence of calciphylaxis in western countries has been reported to vary from 1 to 4% [2], and its prevalence in Japan seems to be much lower. In the present study, we conducted a nationwide questionnaire survey, and about half of the Japanese hemodialysis centers responded to the questionnaire. Assuming that the diagnoses made at the centers were correct, 249 cases of calciphylaxis had been encountered at the centers. We also found reports of 72 sporadic cases in the Japanese literature in the past decade. If we assume that all of these calciphylaxis cases in our survey and the literature were seen within 10 years, the prevalence rate of calciphylaxis in Japan should be less than three cases per 10 000 hemodialysis patients/year. This rate is much lower than the previously reported prevalence rates in western countries. However, in our study, only about half of the hemodialysis centers (1838 centers) responded and a total of 249 potential cases had been encountered at 151 centers. We suspect that the centers that responded were more interested in calciphylaxis. In addition, since ~60% of nephrologists in Japan do not know the disease itself, it is highly likely that calciphylaxis is being overlooked. Based on these results, we speculate that this prevalence rate from our assumptions which are discussed on the above section may underestimate the prevalence rate of calciphylaxis in Japan.

In the present study, we were able to collect 67 cases by conducting a nationwide survey, and the clinical records of 28 definite cases were used for our case–control study. Several risk factors for calciphylaxis in end-stage renal disease patients have been identified in case–control studies in Europe and North America. In 1999, data from 9 calciphylaxis patients were compared with data obtained from a cross-sectional survey of 347 hemodialysis patients [10]. The results of that study showed body mass index (BMI), serum albumin concentration, serum calcium concentration and inorganic phosphate concentration to be significant risk factors for calciphylaxis, and it is interesting that all nine patients were Caucasian. A study by Mazhar *et al.* [11] revealed female gender and erythropoietin therapy to be significant risk factors in addition to the serum albumin level and calcium–phosphate metabolism disturbances. Fine *et al.* [12] conducted a prospective observational study of 36 calciphylaxis patients at a single hemodialysis unit in Canada and reported finding that peritoneal dialysis, calcium carbonate administration, vitamin D analog therapy and diabetes mellitus were all significant risk factors in addition to female gender and phosphate and calcium metabolism disturbances. The prevalence rate of calciphylaxis in their report was 4.5/100 patient-years and much higher than in other reports. However, the report from the same facility in 2008 revealed that the high incidence was attributable to iatrogenic factors [13]. The serum ionized calcium concentration had been used as a marker of calcium metabolism at that facility, and the method of measurement was inappropriate. Because of the inappropriate method and resulting incorrect calcium concentrations, excessive doses of vitamin D analog and calcium salts were administered and presumably induced severe hypercalcemia in the patients. After correcting the method used to monitor calcium metabolism, the prevalence rate decreased dramatically. A case–control study of 47 calciphylaxis patients conducted at the Mayo Clinic was reported in 2007, and it identified BMI [14], steroid use, liver disease, calcium–phosphate product and serum aluminum concentration as significant risk factors for calciphylaxis in end-stage renal disease. In the present study, lower serum albumin levels and higher adjusted calcium concentrations were identified as risk factors by the univariate logistic analysis, the same as in previous studies, whereas adjusted calcium concentrations were found not to be a significant risk factor by the multivariate logistic analysis. Since there were no significant differences in PTH levels and vitamin D therapy between the controls and the cases, the hypercalcemia may have been induced by other factors. In the present study, we asked if the patient had been treated with vitamin D or vitamin D analog but not the dose, and we have no data on medication with calcium-containing salts. It is possible that the therapeutic set point for calcium was higher in the hemodialysis center where the calciphylaxis patients received their medication and that the doses of vitamin D and calcium-containing salt were higher in the calciphylaxis cases.

Warfarin therapy was also identified as a highly significant risk factor for calciphylaxis in our study. It has been suggested that warfarin may play an important role in the development of calciphylaxis by causing changes in the coagulation pathway and vitamin K-dependent bone metabolism, although none of the previous case–control studies mentioned above identified warfarin therapy as a significant risk factor. It was recently reported that warfarin therapy might facilitate vascular calcification in dialysis patients [15] and that warfarin therapy increases the incidence of stroke [16]. However, they were observational studies, and a prospective controlled study will be necessary to determine the usefulness of warfarin therapy in dialysis patients.

Data regarding BMI, dialysis quality (kt/V) and residual renal function, which are considered possible risk factors for the development of calciphylaxis, were not collected in our study. Since our study was based on nationwide surveillance, we chose not to send a complicated data sheet and decided instead to collect the minimum information necessary regarding the calciphylaxis cases to facilitate participation by the hemodialysis centers in our study. In order to collect more detailed information on calciphylaxis, we have inaugurated a Japanese calciphylaxis registry and are going to continue to register for at least 5 years. We hope the registry will make it possible to analyze additional risk factors that were not included in the present study.

In conclusion, the prevalence of calciphylaxis in Japan seems to be much lower than in western countries, suggesting that the incidence of calciphylaxis may vary with racial and geographic factors, although the exact prevalence of

calciphylaxis in chronic hemodialysis patients in Japan cannot be estimated due to the study design. Our study identified warfarin therapy and lower serum albumin concentrations as significant and strong risk factors for the development of calciphylaxis.

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カルシフィラキシス

Calciphylaxis

Key words：カルシフィラキシス，ワルファリン，透析，高カルシウム血症

林　松彦

1. 概念・定義

カルシフィラキシスは，1961年に米国のSelyeにより動物実験を基として提唱された概念である．Selyeはanaphylaxisの研究の延長において，感作因子として副甲状腺ホルモン，ビタミンD関連物質などをラットにあらかじめ投与し，刺激因子として金属塩などを投与した際に皮膚，筋症状を呈することを見いだし，anaphylaxisと対比してcalciphylaxisと名づけたものである[1]．その後，腎不全患者を中心として，皮膚の炎症から壊死に至る，動物実験で生じた皮膚症状と類似した病像を呈する臨床例が報告され，二次性の副甲状腺機能亢進症を伴っていたことから，動物実験におけるカルシフィラキシスと同様の病因によることが推測され，カルシフィラキシスと呼ばれるようになった[2-5]．しかし，病理的な所見は全く異なり，ラットでは皮膚間質の異所性石灰化とそれに基づく病変であるのに対し，臨床例では，小動脈中膜の石灰化，内膜の浮腫状肥厚，更に血栓形成を典型的所見としていることが明らかとなってきた[6]．そこで，カルシフィラキシスという病名は適当ではないとの考えから，calcific uremic arteriolopathyという疾患名が提唱され，米国を中心に広まってきている[7]．一方，一部ではあるが腎不全を伴わない症例も確実に存在しており，uremicと断定することはできないことから，この疾患名が最適ともいえない．

明確な疾患の定義はないが，慢性腎不全患者を中心としてみられる紫斑と有痛性の皮膚潰瘍を臨床的特徴として，病理組織的には患部の小動脈中膜の石灰化，内膜の浮腫状肥厚，血栓形成を特徴とする皮膚疾患をカルシフィラキシスと呼んでいる．

2. 疫　　学

我が国での発症率はこれまで統計がなく不明であったが，平成21年度に，著者らが厚生労働省難治性疾患克服研究事業として行った調査では，透析患者において年間2人/1万人以下と推定された．ただし，疾患に対して極めて認知度が低く，実際にはより多く発症している可能性が考えられる．欧米では1-4％の発症率が報告されている．また，全国調査の際，男女の発症率には差がなかったが，一般透析患者に比べてカルシフィラキシスを発症した症例は年齢層が有意に低かった．

3. 病　　因

1960年代の症例報告では二次性副甲状腺機能亢進症が基礎にあることが示唆された．その後の症例対照研究などの結果では，発症危険因子として高カルシウム血症，高リン血症，肥満，肝疾患，低アルブミン血症，原疾患としての糖尿病腎症，などが同定されている[8-11]．著者らの研究班の調査結果では，低アルブミン血症，高カルシウム血症，高血糖が発症危険因子として同定されたが，最も影響を与えていたのはワルファリンの内服であった．これらの危険因子からカルシフィラキシス発症に至る過程は推測の域を出ていないが，カルシウム・リン代謝の異

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表1　カルシフィラキシス診断基準案(抜粋)

以下の臨床症状2項目と皮膚病理所見を満たす場合，または臨床症状3項目を満たす場合，カルシフィラキシスと診断される．

【臨床症状】

1　慢性腎臓病で透析中，または糸球体濾過率15 mL/min以下の症例
2　周囲に有痛性紫斑を伴う，2カ所以上の皮膚の有痛性難治性潰瘍
3　体幹部，上腕，前腕，大腿，下腿，陰茎に発症する，周囲に有痛性紫斑を伴う皮膚の有痛性難治性潰瘍

【皮膚病理所見】

皮膚の壊死，潰瘍形成とともに，皮下脂肪組織ないし真皮の小～中動脈における，中膜，内弾性板側を中心とした石灰化，および，浮腫性内膜肥厚による内腔の同心円状狭窄所見を認める
（皮膚生検は，症状を増悪させたという報告があり，可能な場合に実施する）

(http://www.dialysis.med.keio.ac.jp/kouroushou/index.html に詳細を掲載)

常が小動脈の石灰化を生じ，小さな外傷などの外的刺激が加わると局所の炎症，そして血栓形成から皮膚壊死に至るという過程が考えられている．また，ワルファリン内服は，凝固因子の局所での変化と，ビタミンK作用拮抗が血管平滑筋細胞を石灰化させるという基礎実験結果もあり，直接発症に関与している可能性が示唆されている．更に，様々な炎症関連タンパク誘導にかかわる情報伝達物質であるNFκBの活性化が中心的役割を果たす可能性も考慮されている[12]．現在では少なくとも，動物実験でSelyeらが提唱した，副甲状腺ホルモンにより感作された状態で，何らかの刺激により皮膚病変が生じるという機序は臨床的なカルシフィラキシスでは否定的である．

4. 病　　態

近位型と遠位型に大別される．近位型は一般に重症であり，下腿，大腿，臀部，躯幹に有痛性の紫斑で始まる皮膚症状が出現し，次第に潰瘍を形成し，拡大，あるいは病変部が増加していく．痛みが極めて強いのが特徴であり，時に硬膜外麻酔が必要となる．潰瘍への感染，更に皮膚病変が広範な場合低栄養も伴い，敗血症などに至って，死亡率は50％前後である．一方，遠位型は，四肢末梢に生じ，手指，足趾に同様の病変が出現する．近位型に比べて予後が良いとされるが，糖尿病性壊疽，あるいは外傷への感染との鑑別は極めて困難である．

5. 診断と鑑別診断

特徴的な皮膚所見を呈する近位型は，カルシフィラキシスという疾患を知っていれば，それほど困難ではない．確定診断は皮膚生検あるいは病巣掻破の際に採取した組織における特徴的病理所見による．問題は，生検を行う場合，生検による創傷が悪化要因になる可能性が指摘されている点である．**表1**に著者らの研究班が提唱している診断基準案を提示するが，この診断基準により少なくとも，全国調査で集めた臨床例はすべて診断可能であった．鑑別診断としては**表2**に挙げた疾患がある．生化学検査所見，血清反応検査などは非特異的炎症所見のみである．

6. 治療と予後

治療の中心は感染対策と，局所の処置である．過去において副甲状腺摘出術が有効であった症例，あるいはシナカルセトの投与が有効であった症例が報告されており[13]，二次性副甲状腺機能亢進症を伴う症例では有用である可能性がある．一方，副腎皮質ステロイド剤の局所，全身投与は同剤自体が危険因子となる可能性が指摘されており，使用すべきではない．最近，チオ硫酸ナトリウムが有効であったとする症例報告がしばしばみられるが[14]，我が国では適応症は

表2 カルシフィラキシスとの鑑別を要する疾患

- ヘパリン起因性血小板減少症(heparin-induced thrombocytopenia: HIT)に伴う皮膚壊死
- ワルファリン潰瘍
- 全身性皮膚硬化症
- nephrogenic systemic fibrosisの初期病変
- コレステロール塞栓
- 蜂窩織炎
- クリオグロブリン血症
- ハイドレアによる皮膚潰瘍
- 抗リン脂質抗体症候群
- 低温熱傷
- 壊死性筋膜炎
- 下肢静脈瘤に伴う潰瘍病変
- 異所性石灰化に伴う皮膚症状

シアン中毒とヒ素中毒となっている点で使用に抵抗がある．副作用としては重篤な代謝性アシドーシスをきたすことがあるが，その際は恐らく透析により改善が期待され，疾患の予後を考えると，十分なinformed consentの後に使用を考慮してもよいであろう．いずれの治療も，疾患の発症頻度が極めて低いことから，前向き無作為試験は実施が事実上不可能である．恐らく，多くの症例を前向きに集めて統計的解析を行うことが最善と考えられ，著者らの研究班ではレジストリーを公開して情報収集を行っている．過去の報告では外科的な患部のdebridment，集中的内科治療，副甲状腺摘出が有効な治療法として同定されている[15]．

疾患の予後は極めて不良で，50％以上が1年以内に死亡したという報告がある．また，著者らの調査でも死亡率は50％を超えている．今後，この疾患への認知度が高まり，早期に集約的な治療を行うことで予後が改善するかを検討していく必要がある．

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腎臓内科学

わが国におけるcacliphylaxisについての全国調査結果と診断規準案策定

National surveillance and diagnostic criteria of calciphyaxis in Japan

Calciphylaxisとは

Calciphylaxis(カルシフィラキシス)は，1960年にSelyeらが動物実験から提唱した概念である．感作因子として副甲状腺ホルモン，ビタミンD関連物質などをラットにあらかじめ投与し，刺激因子として金属塩などを投与した際に皮膚への石灰沈着などの症状を呈する現象を，anaphylaxisと対比してcalciphylaxisと命名したものである[1]．

その後，慢性透析患者を中心として発症する，皮膚の有痛性で多発性・難治性潰瘍を主病変とする疾患が報告され，その発症原因として細小血管の石灰化が主因として考えられたことからcalciphylaxisとよばれるようになった．したがって，動物実験の概念とは異なる病態であり，calcific uraemic atreriolopathyという名称を提唱する研究者もいるが[2]，現在も一般的にはcalciphylaxisとよばれている．

Calciphylaxisは皮膚潰瘍への細菌感染から敗血症などを併発して死亡率が50%を超えることが報告され，欧米での発症率は調査対象などにより異なるが，透析患者の数%に上る可能性も示されている[2]．正確な発症状況については現在，アメリカ，ヨーロッパ，それぞれにおいてレジストリーが作成されており，近い将来，さらに詳細な検討結果が発表されることが期待される．

わが国における全国調査と診断規準案

一方，わが国では本疾患の発症状況についての系統的な調査・研究はなされたことはなく，実態はまったく不明であった．そこでcalciphylaxisの臨床上の重要性から，同疾患の実態把握，病態解明，診断基準作成を目的に著者を研究代表者とした平成21年度厚生労働省難治性疾患克服研究事業「Calciphylaxisの診断・治療に関わる調査・研究」により，はじめて全国調査が実施された．

2009年8月に日本透析医学会所属3,760施設に対して調査票を配布し，151施設から計249例の症例を経験したことが報告された．その結果，症例の性別・年齢分布はわが国の透析患者全体と比べ，男女比はほぼ同等であるが，年齢分布は30代，40代，50代に多い傾向を示した．また，calciphylaxisについて“よく知っている”，“だいたい知っている”と答えた施設は40%にとどまっていた．さらに，67例について病理所見などを含む詳細な臨床情報が集められたが，臨床症状，病理所見よりcalciphylaxisと確定診断できた症例はこのなかの35例であった．これら35例に基づいて表1のような診断規準案を作成し，現在その妥当性を検証中である．

表1　Calciphylaxis診断規準案抜粋

以下の臨床症状2項目と皮膚病理所見を満たす場合，または臨床症状3項目を満たす場合，calciphylaxisと診断される．

臨床症状

1．慢性腎臓病で透析中，または糸球体濾過率15 ml/min以下の症例
2．周囲に有痛性紫斑をともなう，2カ所以上の皮膚の有痛性難治性潰瘍
3．体幹部，上腕，前腕，大腿，下腿，陰茎に発症する，周囲に有痛性紫斑をともなう皮膚の有痛性難治性潰瘍

皮膚病理所見

皮膚の壊死，潰瘍形成とともに，皮下脂肪組織ないし真皮の小～中動脈における，中膜，内弾性板側を中心とした石灰化，および，浮腫性内膜肥厚による内腔の同心円状狭窄所見を認める．

(皮膚生検は，症状を増悪させたという報告があり，可能な場合に実施する)

(http://www.dialysis.med.keio.ac.jp/kouroushou/index.html に詳細を掲載)

おわりに

2010年度以降，レジストリーを作成するとともに，対照研究，前向き観察研究を実施中であり，さらに本症の実態が明らかとなることが期待される．なお，表1の診断規準は抜粋であり，本研究の詳細とともに，以下のホームページを参照されたい(ホームページアドレス http://www.dialysis.med.keio.ac.jp/kouroushou/index.html)．

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NEPHROLOGY – ORIGINAL PAPER

Association of peripheral artery disease and long-term mortality in hemodialysis patients

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Abstract

Background Peripheral artery disease (PAD) is a common complication in hemodialysis patients. The ankle-brachial blood pressure index (ABI) has been widely used to screen for subclinical PAD. In the present study, we investigated the association between ABI and long-term (up to 8.8 years) mortality among hemodialysis patients.

Methods A total of 86 consecutive patients receiving maintenance hemodialysis who underwent an ABI examination between 2001 and 2003 were retrospectively enrolled in this study. Patients with an ABI of less than 0.9 were considered as having PAD; those with an ABI of more than 0.9 in both legs were considered as being free from PAD. We examined the relationship between mortality and several risk factors.

Results During the follow-up period, 43 deaths were recorded. In the univariate regression analysis, the mortality hazard ratio (HR) of patients with PAD was 1.67 (95% confidence interval [CI], 1.18–2.28). Other predictive variables for mortality included male gender, age, and diabetes mellitus ($P = 0.006$, $P = 0.024$, and $P = 0.023$, respectively). A multivariate Cox analysis identified PAD and male gender as independent predictors of mortality ($P = 0.033$ and $P = 0.028$, respectively). The impact of age and diabetes mellitus on mortality was no longer significant in the multivariate analysis.

Conclusion After a relatively long-term observation period, a multivariate analysis indicated that PAD acted independently of other risk factors, including advanced age and the presence of diabetes mellitus. ABI measurements can be used to identify high-risk hemodialysis patients requiring intensive follow-up care.

Keywords Ankle-brachial index · Chronic kidney disease · Long-term outcome · Peripheral artery disease

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Introduction

Peripheral artery disease (PAD) is a common complication in hemodialysis patients. Epidemiological and clinical studies in the general population have demonstrated that the presence of PAD is associated with an increased risk of myocardial infarction and stroke [1, 2]. Limited available data also suggest that PAD is prevalent among hemodialysis patients [3–5] and is associated with poor outcomes [3, 6, 7]. The ankle-brachial blood pressure index (ABI) has been widely used to screen for subclinical PAD and is believed to be strongly correlated with PAD of the lower limbs [8]. A recent study revealed that ABI is an independent and strong predictor of all-cause and cardiovascular mortality in hemodialysis patients [6]. However, the average follow-up period of this previous study was only 2 years [6], and no information is available about ABI and long-term mortality in hemodialysis patients. In the present study, we investigated the association between ABI and long-term (up to 8.8 years) mortality among hemodialysis patients.

Materials and methods

A total of 86 consecutive patients receiving maintenance hemodialysis who underwent an ABI examination between 2001 and 2003 were retrospectively enrolled in this study. Clinical data, including age, gender, duration of hemodialysis therapy, diabetes mellitus complications, and smoking habits, were collected. The clinical follow-up data were obtained from the hospital records. The clinical endpoint was defined as death from any cause. Patients with an ABI of less than 0.9 were considered as having PAD (PAD group); those with an ABI of more than 0.9 in both legs were considered as being free from PAD (non-PAD group) [9]. We compared the clinical data among the PAD group and the non-PAD group and examined the relationship between mortality and several risk factors. Among 41 patients who were more than 60 years old, we also compared the survival rate between the PAD group and the non-PAD group.

ABI was determined in all the patients using an ABI-form (Colin, Japan), which simultaneously measures bilateral arm and ankle blood pressures (brachial and posterior tibial arteries, respectively) using the oscillometric method. The blood pressure was measured after a dialysis session and after the patients had rested in a supine position for at least 5 min. The ABI was calculated using the ratio of the ankle systolic pressure divided by the arm systolic pressure. The systolic pressure of the arm without dialysis access and the lower value of the ankle pressure were used for the calculation.

Statistical analysis

All data were expressed as means ± SD. The Student *t* test was used for comparisons between continuous variables. The chi-square or Fisher exact probability test was applied for categorical data. Univariate and multivariate analyses used the Cox proportional hazards model. The survival curves were estimated using the Kaplan–Meier method followed by a log-rank test. All statistical calculations were performed using JMP 5.1 software. *P* values less than 0.05 were considered to denote statistical significance.

Results

The patient background characteristics are shown in Table 1. Among the 86 patients, 19 patients had PAD (22.1%). The patients in the PAD group were significantly older (65.2 ± 8.3 years) than those in the non-PAD group (58.3 ± 8.6 years, $P = 0.0027$). The percentage of patients with diabetes mellitus was also higher in the PAD group (42.1%) than in the non-PAD group (13.4%, $P = 0.0099$). No differences in the duration of hemodialysis and the smoking habits were observed between the two groups.

During the follow-up period, 43 deaths were recorded. Table 2 shows a Cox proportional hazards analysis of the covariates for predicting mortality. In the univariate regression analysis, the hazard ratio (HR) of patients with PAD was 1.67 (95% confidence interval [CI], 1.18–2.28). Other predictive variables for mortality included male gender, age, and diabetes mellitus ($P = 0.006$, $P = 0.024$, and $P = 0.023$, respectively). The multivariate Cox analysis identified PAD and male gender as independent predictors of mortality ($P = 0.033$ and $P = 0.028$, respectively). The impact of age and diabetes mellitus on mortality was no longer significant in the multivariate analysis.

Table 1 Background characteristics of the study participants

	Total	PAD group	Non-PAD group	*P* value
Number *n* (male, % male)	86 (60, 69.8%)	19 (14, 73.7%)	67 (46, 68.7%)	NS
Age (years)	59.8 ± 9.0	65.2 ± 8.3	58.3 ± 8.6	0.0027
Duration of hemodialysis (years)	15.1 ± 10.1	16.7 ± 9.9	14.6 ± 10.1	NS
Diabetets mellitus *n*, (%)	17 (19.8%)	8 (42.1%)	9 (13.4%)	0.0099
Smoking *n*, (%)	18 (20.9%)	3 (15.8%)	15 (22.4%)	NS

Table 2 Cox proportional hazards regression analysis for mortality

Parameters	Univariate		Multivariate	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
PAD (Y)	1.63 (1.18–2.28)	0.004	1.58 (1.04–2.38)	0.033
Gender (male)	1.67 (1.15–2.61)	0.006	1.55 (1.05–2.46)	0.028
Age (per year)	1.04 (1.01–1.08)	0.024	1.02 (0.98–1.06)	0.305
Duration of dialysis (per 1 year)	0.98 (0.95–1.01)	0.208	0.99 (0.95–1.02)	0.446
Diabetets mellitus (Y)	1.48 (1.06–2.02)	0.023	1.15 (0.76–1.71)	0.504
Smoking (Y)	1.19 (0.82–1.65)	0.338	1.25 (0.84–1.80)	0.263

Table 3 Background characteristics of study participants over the age of 60 years

	Total	PAD group	Non-PAD group	*P* value
Number *n* (male, % male)	41 (30, 73.2%)	14 (10, 71.4%)	27 (20, 74.1%)	NS
Age (years)	67.4 ± 5.0	68.7 ± 6.4	66.7 ± 4.0	NS
Duration of hemodialysis (years)	12.3 ± 8.7	13.0 ± 8.9	11.9 ± 8.7	NS
Diabetets mellitus *n*, (%)	10 (24.4%)	8 (57.1%)	2 (7.4%)	0.001
Smoking *n*, (%)	5 (12.2%)	1 (7.1%)	4 (14.8%)	NS

The background data for hemodialysis patients over the age of 60 years at the start of the observation period are shown in Table 3. Among the 41 patients, 14 patients had PAD. The patients' age was not significantly different between the two groups. The rate of diabetes mellitus complications was higher in the PAD group (57.1%) than in the non-PAD group (7.4%, $P = 0.0001$). Figure 1 shows a comparison of survival curves for patients with PAD and those without PAD. Even though the age was similar in the two groups, the curves showed a statistically significant difference between the two groups ($P = 0.0289$). Patient survival rate at 8 years was 28.6% in the PAD group and 61.6% in the non-PAD group.

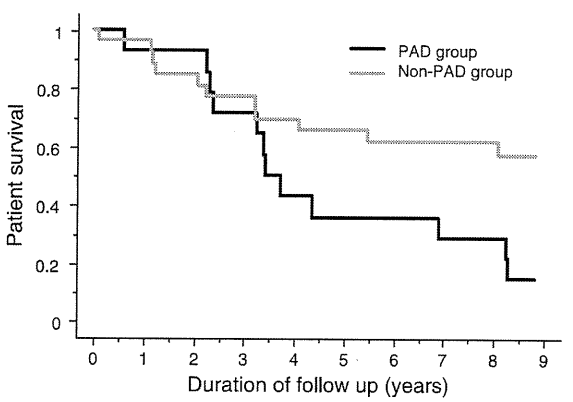

Fig. 1 Survival probabilities of hemodialysis patients with or without PAD who are over the age of 60 years. Log-rank test, $P = 0.0289$

Discussion

The prevalence of PAD in our center was 22.1%. This prevalence was higher than that reported by the United States Renal Data System (USRDS) (15%) and similar to that reported by the HEMO Study (23%) in patients undergoing maintenance hemodialysis [4, 10]. This value is much higher than previously published data in an Asian population of type 2 diabetes patients without chronic kidney disease (10%) and almost the same as that reported for Taiwanese peritoneal dialysis patients (19.6%) [11, 12]. In dialysis patients, both atherosclerosis (mainly affecting the intima of the arteries) and arteriosclerosis (affecting predominantly the media of large- and middle-sized arteries diffusely) are highly prominent. Arteriosclerosis, characterized by reduced arterial compliance (i.e., reduced elasticity of the arteries), is due to increased fibrosis, loss of elastic fibers, and extensive vessel wall calcification [13]. The transformation of vascular smooth muscle cells into chondrocytes or osteoblast-like cells seems to be a key element in vascular calcification pathogenesis, in the context of passive calcium and phosphate deposition due to abnormal bone metabolism and impaired renal excretion [14].

Some studies have reported conventional risk factors for PAD in hemodialysis patients, including advanced age, white race, male gender, diabetes mellitus, coronary artery disease, cerebrovascular disease, smoking, and left ventricular hypertrophy [4, 15]. Dialysis-specific factors included the duration of dialysis, a malnourished status, hypoalbuminemia, disorders of mineral metabolism, and hypoparathyroidism [4, 15, 16]. In our study, PAD was significantly correlated with advanced age and diabetes in hemodialysis patients, similar to the results of previous reports [4, 15]. The duration of hemodialysis tended to be longer in PAD patients than in non-PAD patients but was not statistically significant. This difference may be a result of the rather small number of examined patients. Other associations with PAD could not be evaluated because of the lack of data.

Cardiovascular disease is the leading cause of death in both chronic kidney disease and peritoneal dialysis/hemodialysis patients [13, 16]. Vascular disease prevention in these patients is therefore important to reduce the incidence of cardiovascular events and the high morbidity and mortality [16]. An important issue in the management of patients with PAD is the elevated risk of cardiovascular disease, stroke, and premature death among patients with PAD of the lower limbs [2, 14, 17]. ABI is a simple, reliable tool that can be used to diagnose the existence and severity of PAD. ABI was determined using the ABI-form (Colin, Japan), which simultaneously measures the arm and ankle blood pressures using an oscillometric method. This automatic device allowed us to measure the ABI quickly, non-invasively, and reproducibly. Several recent investigations have also shown an association between an abnormal ABI and cardiovascular morbidity and mortality [18, 19]. As the results of our relatively long-term observation period (maximum of 8.8 years) showed, PAD was an independent risk factor for mortality. The survival of hemodialysis patients older than 60 years of age was 28.6% in the PAD group and 61.6% in the non-PAD group at 8 years. The multivariate analysis indicated that PAD acted independently of the other risk factors, including an advanced age and diabetes mellitus. Thus, the measurement of ABI effectively identified high-risk hemodialysis patients, a target population requiring intensive follow-up. Previously, multiple conventional risk factors for mortality in hemodialysis patients were analyzed [6]. Although diabetes mellitus was a strong predictor in the univariate analysis, it did not have a statistically significant impact on mortality in the multivariate analysis. These results are similar to those obtained in the present study, suggesting that the impact of diabetes mellitus might be mediated by atherosclerotic vascular disease, represented by a low ABI [6]. In our study, a male gender was another independent risk factor for mortality, similar to the results of a previous report [6].

Our study had several limitations. First, the study was a retrospective examination of patients evaluated at a single institution, and there were differences in care, including medications, over the course of the study. Second, the associations of other factors with PAD could not be evaluated because of a lack of data.

Conclusions

PAD is strongly correlated with advanced age and diabetes in hemodialysis patients. After a relatively long-term observation period, a multivariate analysis indicated that PAD acted independently of other risk

factors, including age and diabetes mellitus. ABI is a simple, reliable tool that can be used to diagnose the existence and severity of PAD. Thus, ABI measurements can be used to identify high-risk hemodialysis patients requiring intensive follow-up care.

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Comparison between Whole and Intact Parathyroid Hormone Assays

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Abstract: The standard measurement of parathyroid hormone (PTH) is the intact PTH (iPTH) assay, which is used for approximately 90% of Japanese dialysis patients. The iPTH assay reacts not only with 1–84 PTH, but also with large truncated fragments of non-1–84 PTH, including 7–84 PTH. On the other hand, the whole PTH assay is specific for 1–84 PTH. The aim of the current study was to define the validity of both whole and intact PTH assays. A total of 738 hemodialysis patients were enrolled from twelve dialysis services. The serum PTH level was evaluated by both intact and whole PTH assays simultaneously. Non-1–84 PTH was determined by subtracting the whole PTH value from that of the intact PTH assay. The median level of whole PTH was 121 pg/mL, and that of iPTH was 210 pg/mL. The whole PTH assay had a very high correlation with the iPTH assay ($r = 0.870, P < 0.001$). For 43 out of 738 patients (5.8%) the value for intact PTH—whole PTH was <0. Both assays significantly correlated with non-1–84 PTH ($P < 0.001$), while the iPTH assay, particularly, had a very high correlation with non-1–84 PTH ($r = 0.791$). As a whole, 18% of the total population was misclassified into a different Japanese guideline category. Stratified by Japanese guideline classifications, 28% of patients within an iPTH target range were misclassified. Using Bland–Altman plot analysis, as the serum PTH level increased, there was a large difference between two assays. Both PTH assays correlate strongly, although the whole PTH assay may be more useful for precise evaluation of PTH function than the iPTH assay. **Key Words:** Intact parathyroid hormone, non-1–84 parathyroid hormone, N-parathyroid hormone, whole parathyroid hormone.

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Secondary hyperparathyroidism is commonly observed in dialysis patients. Measurement of parathyroid hormone (PTH) values is used to assess the severity of hyperparathyroidism, to decide when to start a vitamin D receptor activator or cinacalcet, and

to monitor the effectiveness of treatment. The standard measurement of PTH is the intact PTH (iPTH) assay, which was developed in late 1980s. In Japan, the iPTH assay was used in approximately 90% of all dialysis patients in the data of Japanese Renal Data Registry 2009, although, the iPTH assay was shown to react not only with 1–84 PTH but also with large truncated fragments of non-1–84 PTH, including 7–84 PTH (1–4). In humans, 20–60% of PTH measured with the iPTH assay corresponds to non-1–84 PTH (1,3–5). In dialysis patients, the percentage of non-1–84 PTH calculated by the iPTH assay is generally greater than normal people (1–6). The whole PTH assay has been shown to be specific for 1–84 PTH. Serum concentrations of large, truncated fragments of non-1–84 PTH can be determined by subtracting the whole PTH value from that measured with the iPTH assay (7,8). In parathyroidectomized rats, a 7–84 PTH infusion, which is a main fragment of non-1–84 PTH, was shown to inhibit a calcemic action of simultaneously infused 1–84 PTH (7). These results suggest that large, truncated PTH fragments similar to 7–84 PTH may be an important cause of skeletal resistance to 1–84 PTH in uremia. In a study of hemodialysis patients, the 1–84 PTH/non-1–84 PTH ratio was less than 1 only in those patients with low bone turnover (8). This result also suggests that large carboxy-terminal fragments may antagonize the skeletal effects of 1–84 PTH. Moreover, the 7–84 PTH fragment, like other carboxy-terminal fragments, binds only to the carboxyl PTH receptor and does not affect the binding of 1–84 PTH to the PTH related peptide (PTHrP) receptor (9–11). Thus, it would appear that the 7–84 PTH fragment may decrease the calcemic action of 1–84 PTH through its interaction with the carboxyl PTH receptor. In order to assess the severity of hyperparathyroidism accurately, it is important to understand the characteristics and the differences of both whole and intact PTH assays.

The aim of the current study was to define the validity of both whole and intact PTH assays by assessing: (i) the comparison of distribution; (ii) the correlation between both assays; (iii) the association of both assays with patient characteristics; and (iv) the comparison between whole PTH and calculated 1–84 PTH levels.

PATIENTS AND METHODS

Patient selection

A total of 738 hemodialysis patients were enrolled in Japan from twelve hemodialysis clinics in five prefectures between June 2000 and May 2006. These associated clinics included: Tokai University School of Medicine, Sekisinkai Kawasaki Clinic, Dai2-Rokushima Clinic, Sekishin Clinic, Japan Red Cross Musashino Hospital, Nakano Clinic, Kyonan Clinic, Suda Clinic, Tokyo Women's Medical University, Mihama Narita Clinic, Matsushita-Kai Akebono Clinic, and Sumiyoshi Clinic Hospital. Entry criteria included having been hemodialysis patients in the preceding three months, the ability to provide informed consent for participation, and being of an age greater than 19 years.

Data collection and PTH assays

Blood used for PTH analyses was collected from the study participants as part of a special lab specimen draw. The serum PTH level was evaluated by both intact and whole PTH assays simultaneously. An EDTA-containing tube was collected before hemodialysis, kept on ice for 1 h, and immediately centrifuged at 1000 *g* for 10 min, separated and refrigerated. PTH has been shown to be stable for 72 h in refrigerated EDTA plasma (12). Specimens were stored at –20°C until they were thawed for analysis at DS Pharma Biomedical (Osaka, Japan).

A single incubation step immunoradiometric assay specific for whole PTH (1–84) was developed and optimized with the previously-mentioned assay reagents. Briefly, 200 μL of assay standards, controls, and patient samples were pipetted into appropriately labeled 12 mm × 7.5 mm polypropylene test-tubes. One hundred microliters of ^{125}I-labeled PTH-specific antibody tracer solution and one goat anti-PTH (39–84) polyclonal antibody-coated bead were added to all test-tubes (1–4). The immunochemical reaction was conducted at room temperature with shaking at 170 rpm for 18–22 h. During this assay incubation period, the immunochemical reaction forming the sandwich of (solid-phase goat anti-PTH (39–84) antibody)—(whole PTH (1–84))—(^{125}I-goat anti-PTH antibody) takes place in correlation with the amount or concentration of whole PTH (1–84) in the test sample. All beads in the test tubes, except the total count tube, were washed with the wash solution, and the radioactive signals from each bead were counted for 1 min using a gamma scintillation counter. The data were processed and calculated using nonlinear regression data reduction software.

Other laboratory measures

Other laboratory measures included measures of serum calcium, phosphate, albumin, alkaline phosphatase, total cholesterol, and HDL-cholesterol from dates that were closest available to the special lab draw date. Serum calcium was adjusted for albumin

using the formula: adjusted calcium = measured calcium – ((4.0 – serum albumin in g/dL)) (13). All calcium values reported and used in this analysis were corrected using the above formula. PTH values were log-transformed in some analyses due to their non-normal distributions.

Statistical analysis

Data are expressed as the mean ± SD. All statistical analyses were conducted using the DOCTOR SPSS II 18.0 program (SPSS Japan, Tokyo, Japan). Differences between groups were calculated using the Mann–Whitney *U*-test, the χ^2-test or ANOVA, where appropriate. Correlation coefficients were calculated by the Pearson method. The comparison of ranks between the whole and intact PTH values was performed using Student's *t*-test. A P value <0.05 was considered significant.

RESULTS

Baseline characteristics and laboratory data

As shown in Table 1, we studied 738 patients (58% male gender) with end-stage renal failure from five dialysis units. All patients underwent chronic hemodialysis treatment three times weekly. The median age of the patients was 60.0 years (range 9–103, mean 58.8 ± 12.6 years). The median vintage of hemodialysis was 132 months (range 2–1238, mean 145 ± 102 months). The underlying renal diseases were: chronic glomerulonephritis, 66%; diabetic nephropathy, 17%; hypertensive nephrosclerosis, 4%; and others, 13%. Of the patients, 24% had a history of cardiovascular disease. The ratio of use of vitamin D receptor activators (i.v. and oral) was 65%. The use of 3.0 mEq/L containing dialysate was 79% of the patients.

At entry, serum corrected calcium and phosphate values were 9.6 ± 0.9 mg/dL and 5.7 ± 1.4 mg/dL, respectively. Serum albumin and alkaline phosphatase concentrations were 3.8 ± 0.4 g/dL and 258 ± 118 IU/L, respectively. At baseline, serum total cholesterol and HDL-cholesterol values were 158 ± 35 mg/dL and 50 ± 16 mg/dL, respectively. The body mass index and Kt/V were 21.1 ± 3.2 kg/m^2 and 1.48 ± 0.28, respectively.

Distribution of serum whole and intact PTH levels

The median serum whole PTH level was 121 pg/mL (range 2–888, mean 164 ± 143 pg/mL; Table 1). The interquartile range of whole PTH was 63–223 pg/mL. The median iPTH was 210 pg/mL (range 3–1436, mean 259 ± 216 pg/mL), and the interquartile range was 111–346 pg/mL. The distribution of whole and iPTH was revealed in Figure 1. Both whole PTH and iPTH assays showed log-normal distribution. There were 224/738 (30.4%) patients with a whole PTH level between 35–105 pg/mL, which is the target range of the Japanese guidelines. Those with an iPTH level between 60–180 pg/mL numbered 221 of 738 (29.9%). Those with whole PTH level <35 pg/mL were 98/738 (13.3%), and those with an iPTH level <60 pg/mL numbered 106/678 (14.4%).

TABLE 1. *Patient baseline characteristics*

Characteristics	
N	738
Age (years)	59 ± 13
Gender (% male)	58
Duration of hemodialysis (months)	145 ± 102
Etiology of renal failure (%)	
Glomerulonephritis	66
Diabetes mellitus	17
Hypertension	4
Other	13
Comorbid conditions (%)	
Diabetes mellitus	18
Cardiovascular disease	24
Fracture	6
History of parathyroidectomy or PEIT	24
Treatment (%)	
Use of an anti-hypertension drug	74
Use of oral or injectable VDRA	65
Use of 3.0 mEq/L Ca-containing dialysate	79
Baseline laboratory tests	
Albumin (g/dL)	3.8 ± 0.4
Calcium (mg/dL)	9.4 ± 0.9
Corrected calcium (mg/dL)	9.6 ± 0.9
Phosphate (mg/dL)	5.7 ± 1.4
Alkaline phosphatase (U/L)	258 ± 118
Whole PTH (pg/mL)	164 ± 143
Median	121
Range	2–888
Intact PTH (pg/mL)	259 ± 216
Median	210
Interquartile range	3–1436
Total cholesterol (mg/dL)	158 ± 35
HDL cholesterol (mg/dL)	50 ± 16
Body mass index (kg/m^2)	21.1 ± 3.2
Kt/V	1.48 ± 0.28
nPCR (g/kg/day)	1.26 ± 0.49

HDL high-density lipoprotein; nPCR, normalized protein catabolic rate; PEIT, percutaneous parathyroid gland ethanol injection therapy; PTH, parathyroid hormone; VDRA, vitamin D receptor activator.

Correlation between serum whole PTH and iPTH levels

The association between whole and intact PTH is shown in Figure 2. The whole PTH assay had a very high correlation with iPTH, as shown below:

$$\text{Intact PTH} = \text{Whole PTH} \times 1.59$$
$$\text{Whole PTH} = \text{Intact PTH} \times 0.63$$
$$(r = 0.870,\ P < 0.001)$$

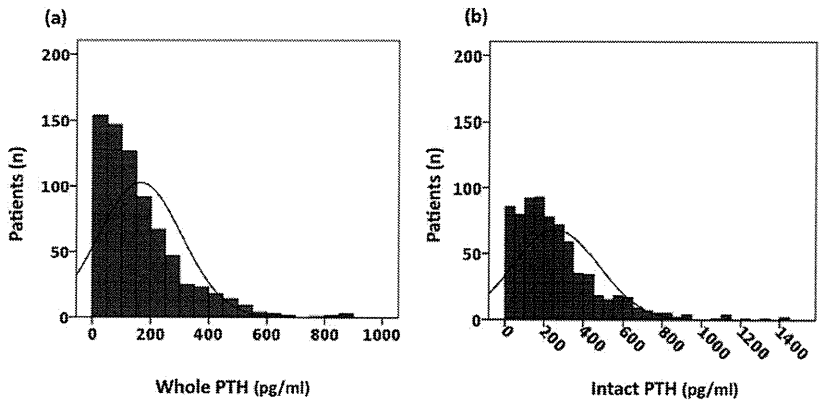

FIG. 1. Distribution of serum (a) whole parathyroid hormone (PTH), and (b) intact PTH levels. The median serum whole PTH level was 121 pg/mL (range 2–888, mean 164 ± 143 pg/mL). The median intact PTH was 210 pg/mL (range 3–1436, mean 259 ± 216 pg/mL). Both assays showed log-normal distribution.

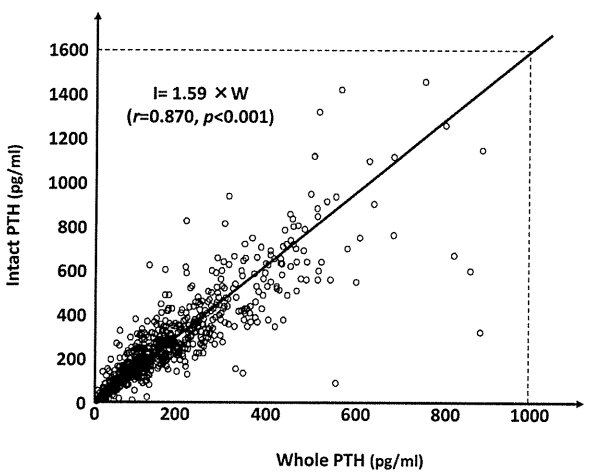

FIG. 2. Correlation between serum whole parathyroid hormone (PTH) and intact PTH levels. The whole PTH assay had a very high correlation with the intact PTH assay ($r = 0.870$, $P < 0.001$).

Then, we divided patients into three categories according to serum iPTH level: ≤60 pg/mL, 61–180 pg/mL, and >180 pg/mL (Fig. 3). In each category, the correlation coefficients were different. The correlation coefficient with iPTH below 60 pg/mL was 1.29, which was obviously lower than 1.59. As the serum PTH level increased, the correlation coefficient between whole and intact PTH levels increased.

Non-1–84 PTH

Serum concentrations of large, truncated fragments of non-1–84 PTH can be determined by subtracting the whole PTH value from that measured with the iPTH assay (7,8). Theoretically, the serum PTH level measured with the iPTH assay is higher than that with the whole PTH assay; however, rare exceptions to this rule have been reported in some

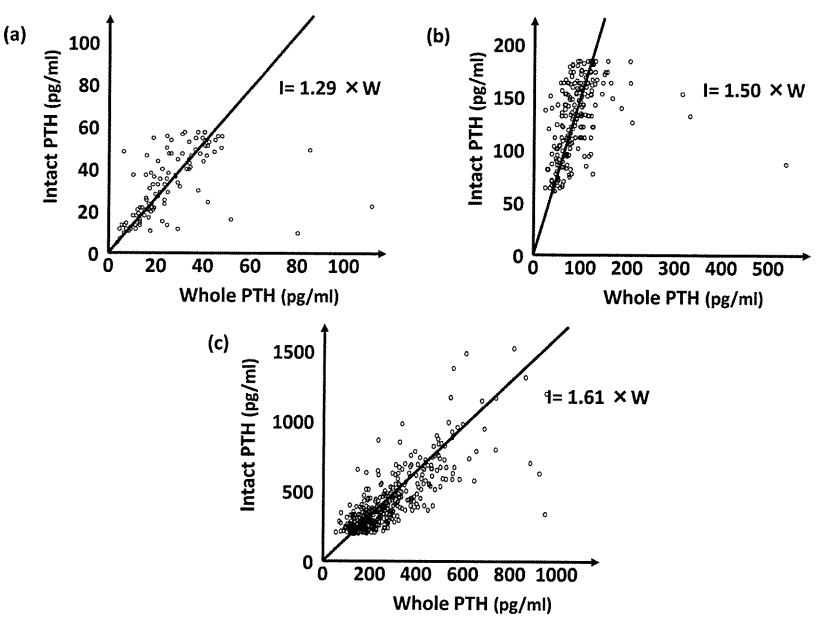

FIG. 3. Correlation between both assays in each intact parathyroid hormone (PTH) level: (a) ≤60 pg/mL; (b) 61–180 pg/mL; and (c) >180 pg/mL. In patients with low PTH levels (<60 pg/mL), the correlation coefficient was comparably low.

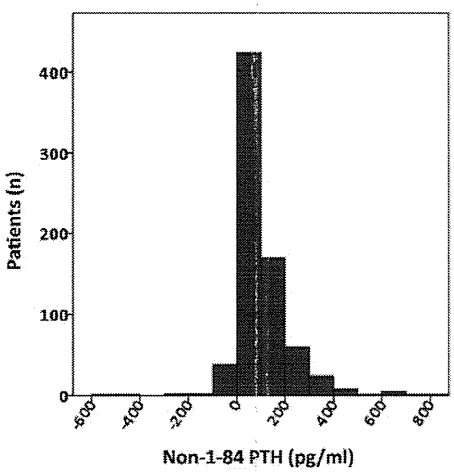

FIG. 4. Distribution of serum non-1–84 parathyroid hormone (PTH) level. There were 43/738 patients (5.8%) with (intact PTH – whole PTH) < 0.

patients, associated with N-PTH overproduction (14–16). As shown in Figure 4, the patients with (iPTH—whole PTH) < 0 numbered 43/738 (5.8%). The association between non-1–84 PTH and each assay is shown in Figure 5. Both assays were significantly correlated with non-1–84 PTH ($P < 0.001$). In particular, the iPTH assay had a very high correlation with non-1–84 PTH ($r = 0.791$).

Association of whole PTH, iPTH, and non-1–84 PTH with patient characteristics

The patients who were younger than 65 years and did not have diabetes mellitus, high calcium, high phosphate, or high alkaline phosphatase had higher whole and intact PTH levels, significantly (Table 2). Among these, serum calcium, phosphate, and alkaline phosphatase levels were significantly related with non-1–84 PTH.

In multivariate linear regression analysis, patient characteristics associated with greater whole PTH levels included male gender, dialysate calcium 3.0 mEq/L versus 2.5 mEq/L, higher corrected calcium, higher phosphate, and higher alkaline phosphatase levels (Table 3). These results were all the same as the iPTH assay. Patient characteristics associated with higher non-1–84 PTH levels included dialysate calcium 3.0 mEq/L, higher serum albumin, higher calcium, higher phosphate, and higher alkaline phosphatase levels.

Comparison between whole PTH and calculated 1–84 PTH levels

When the 1–84 fraction was calculated using 63% of the iPTH, 18% (131/738) of the total population was misclassified into a different Japanese guideline category. The percentage agreement was 82%. Stratified by Japanese guideline classifications, 24% (27/114) of those below target range were misclassified, while 28% (60/214) and 11% (44/410) of those within or above target range, respectively, were misclassified. The Bland–Altman plot, showing the difference between whole PTH and calculated 1–84 PTH plotted against the mean of the two values, reveals that at low PTH levels, there are a few significant differences between the two assays, but that, as the PTH levels increase, there is a large difference between the two measures (Fig. 6).

DISCUSSION

The first objective of the present study was to elucidate a difference between whole and intact PTH assays. At first, we investigated the distribution of both assays and, as shown in Figure 1, both assays revealed a log-normal distribution. Patients whose PTH level was within the Japanese target range were about 30% in both assays. In accordance with some previous reports (17–20), there was a strong correlation ($r = 0.870$) between both assays in the present

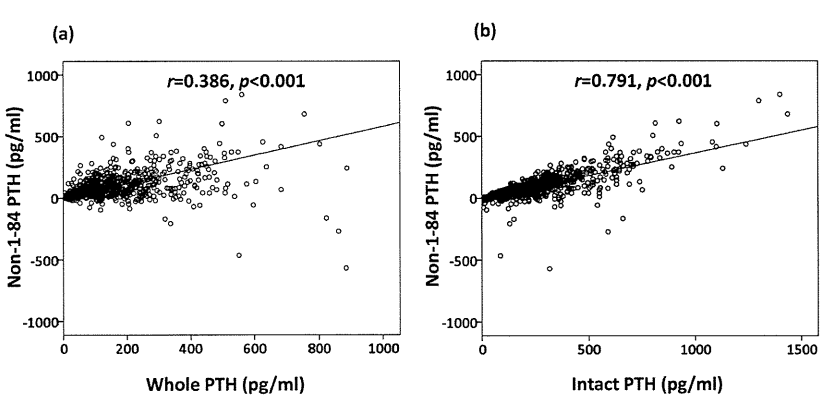

FIG. 5. Correlation between non-1–84 parathyroid hormone (PTH) and (a) the whole PTH and (b) intact PTH assays. Both assays were significantly correlated with non-1–84 PTH ($P < 0.001$). In particular, the intact PTH assay had a very high correlation with non-1–84 PTH ($r = 0.791$).

TABLE 2. *Whole parathyroid hormone (PTH), intact PTH and non-1–84 PTH by patient characteristics*

Characteristics	Whole PTH (pg/mL)		Intact PTH (pg/mL)		Non-1–84 PTH (pg/mL)	
	Median (IQR)	*P* value	Median (IQR)	*P* value	Median (IQR)	*P* value
Age						
<65 years ($N = 498$)	134 (70–238)	<0.01	224 (120–360)	<0.01	75 (27–147)	0.06
≥65 years ($N = 240$)	105 (43–204)		181 (85–280)		64 (23–110)	
Gender						
Male ($N = 431$)	130 (63–233)	0.18	219 (110–360)	0.32	68 (24–134)	0.85
Female ($N = 307$)	109 (60–212)		201 (102–336)		75 (27–137)	
Hemodialysis vintage						
<132 months ($N = 369$)	115 (54–209)	0.05	196 (101–318)	0.22	71 (31–133)	0.91
≥132 months ($N = 369$)	127 (70–239)		220 (119–355)		70 (19–142)	
Diabetes						
Absent ($N = 603$)	127 (66–237)	<0.01	221 (117–353)	<0.01	75 (24–144)	0.07
Present ($N = 135$)	101 (52–172)		160 (93–266)		58 (28–101)	
Corrected calcium						
<9.4 mg/dL ($N = 359$)	110 (59–204)	<0.01	180 (105–302)	<0.01	58 (25–108)	<0.01
≥9.4 mg/dL ($N = 379$)	133 (64–237)		242 (110–370)		86 (25–162)	
Phosphate						
<5.6 mg/dL ($N = 351$)	101 (49–177)	<0.01	180 (88–285)	<0.01	60 (22–115)	<0.01
≥5.6 mg/dL ($N = 387$)	150 (79–272)		244 (130–420)		77 (30–162)	
Alkaline phosphatase						
<234 U/L ($N = 359$)	104 (52–196)	<0.01	176 (91–290)	<0.01	58 (18–113)	<0.01
≥234 U/L ($N = 353$)	141 (76–245)		240 (140–386)		80 (34–162)	

IQR, interquartile range.

study. The correlation coefficient was 1.59, which was almost the same as that of the Japanese guidelines (21). When we divided patients into three categories according to the serum iPTH level, the correlation coefficient with iPTH below 60 pg/mL was 1.29, which was obviously lower than 1.59. This difference of the coefficient would be due to N-PTH overproduction or some PTH fractions, such as 7–84 PTH fragment. During hypercalcemia, the proportional decrease in carboxyl terminal fragments is less than that of 1–84 PTH (22–24). In hemodialysis patients, the percent of non-1–84 PTH was shown to directly correlate with the predialysis serum calcium concentration, with an increase in the serum calcium concentration associated with a reduction in the ratio of 1–84 PTH/non-1–84 PTH (7,8). These results suggest that hypercalcemia or calcium load would induce the fragmentation of 1–84 PTH and lead to the increase of the non-1–84 PTH value.

In the category with iPTH <60 pg/mL, iPTH is considered to be underestimated. Twenty-four percent of the low iPTH category showed serum whole PTH levels within the Japanese target range (35–105 pg/mL). In the category with iPTH 61–180 pg/mL, 22% of the patients showed high whole PTH levels (>105 pg/mL). On the other hand, in the high iPTH category, 11% of the patients revealed serum whole PTH levels within the Japanese target range (35–105 pg/mL). The distribution of both assays was alike and the correlation between both assays was high, although there were a considerable number of misclassifications.

We elucidated an association between each PTH assay and the patient characteristics. In multivariate

TABLE 3. *Adjusted differences in whole parathyroid hormone (PTH), intact PTH and non-1–84 PTH by patient characteristics*

Characteristics	Whole PTH (pg/mL)		Intact PTH (pg/mL)		Non-1–84 PTH (pg/mL)	
	β	P value	β	P value	β	P value
Age, per 10 years	−0.06	0.15	−0.03	0.51	0.21	0.58
Female vs. male	−0.07	0.04	−0.06	0.09	−0.21	0.57
Hemodialysis vintage, per 10 months	0.02	0.60	0.003	0.94	−0.17	0.66
Diabetes mellitus vs. no diabetes mellitus	−0.05	0.22	−0.04	0.29	−0.16	0.68
Dialysate calcium 3.0 mEq/L vs. 2.5 mEq/L	0.09	0.01	0.11	<0.01	0.87	0.02
Albumin, per g/dL	−0.05	0.21	0.05	0.20	0.18	<0.01
Corrected calcium, per mg/dL	0.12	<0.01	0.13	<0.01	0.09	0.03
Phosphate, per mg/dL	0.27	<0.01	0.28	<0.01	0.19	<0.01
Alkaline phosphatase, per U/L	0.19	<0.01	0.23	<0.01	0.20	<0.01