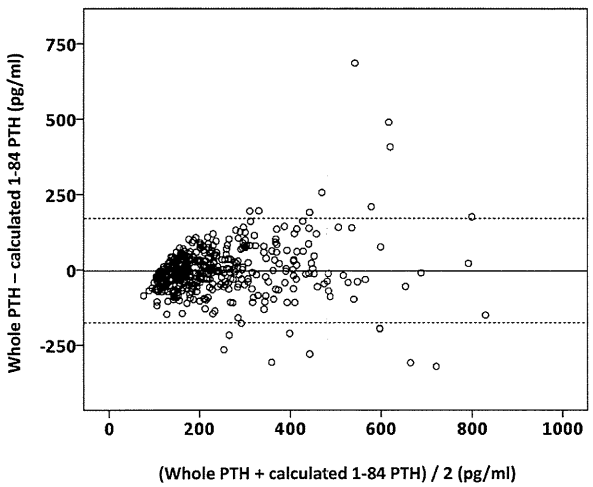

FIG. 6. Bland–Altman plot of whole parathyroid hormone (PTH) versus calculated 1–84 PTH. At low PTH levels, there are a few significant differences between two assays; however, as the PTH levels increase, there is a large difference between the two measures (calculated 1–84 PTH = intact PTH × 0.63).

analysis using a linear regression model, the patients with both greater whole and intact PTH levels include higher dialysate calcium concentrations and higher serum calcium levels. Hypercalcemia usually inhibits PTH secretion. Elevation of the serum calcium level with a high PTH state was considered due to medical therapy, such as with vitamin D or calcium carbonate. The serum non-1–84 PTH level significantly correlated with dialysate calcium 3.0 mEq/L, higher serum albumin, higher calcium, higher phosphate, and higher alkaline phosphate levels. These results indicated that a high serum calcium level or calcium overload would cause an increase of non-1–84 PTH production.

The amount of 1–84 PTH was calculated using 63% of the iPTH. 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 the iPTH target range were misclassified. The Bland–Altman plot (Fig. 6), showing the difference between whole PTH and calculated 1–84 PTH (=iPTH × 0.63) plotted against the mean of the two values. There is not a systematic bias in this comparison. As the PTH levels increase, there is a large difference between the two measures. There were many outliers in the whole PTH side (the upper part of the graph). It is probably due to N-PTH overproduction. Some outliers in the calculated 1–84 PTH side (the lower part of the graph) would reveal PTH fragmentations.

There are several limitations in the present study. The first is that we did not have data for mortality, onset of cardiovascular disease, or fracture. If possible, we should elucidate an association between the serum PTH level and mortality or fracture in each PTH assay. Melamed et al. (25) had reported that an elevated 1–84 PTH value was significantly associated with an increased risk of death, whereas iPTH was not significantly associated with mortality. Lehmann et al. (26) took bone biopsies from 132 patients with chronic kidney disease in stages 3–5 and evaluated the association of bone histomorphometry with the iPTH and Bio-Intact PTH assays. Both assays effectively identified patients with reduced bone turnover. There was no difference between both assays. According to these reports, the difference of the PTH assays may not have much influence on the risk of either mortality or fracture.

In summary, in this cohort of dialysis patients, although the distribution of both assays was alike and the correlation between both assays was high, there were some misclassifications. Eighteen percent of the total population was misclassified into a different Japanese guideline category. As the PTH levels increased, there was a large difference between the two measures. In conclusion, both PTH assays were strongly correlated, although, the whole PTH assay may be more useful for precise evaluation of PTH activity than the iPTH assay.

Conflict of interests: The authors have no conflict of interests.

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ORIGINAL RESEARCH

Nonenzymatic Cross-Linking Pentosidine Increase in Bone Collagen and Are Associated with Disorders of Bone Mineralization in Dialysis Patients

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Abstract Disorders of bone and mineral metabolism are common complications in chronic kidney disease (CKD) patients and lead to significantly increased fracture risk, morbidity, and mortality of cardiovascular disease due to ectopic calcifications, contributing to a worsening prognosis. Bone strength is determined by not only bone mineral density but also bone quality, which is dependent on bone collagen cross-links. Collagen cross-links are classified into enzymatic immature and mature types and nonenzymatic advanced glycation end products (AGEs). Pentosidine is well established as one of the AGEs that accumulates markedly in CKD patients. The chemistry, function, and clinical relevance of cross-links have been revealed, whereas bone quality and the relationship with bone mineralization in CKD patients are not clear. We performed transiliac bone biopsies on 22 dialysis patients (mean age 56 ± 9 years) with severe secondary hyperparathyroidism and measured cross-links by evaluating bone histomorphometry. Cross-links data were compared with age-matched non-CKD subjects (mean age 58 ± 8 years, $n = 17$). Enzymatic collagen cross-links were formed to a similar extent compared with non-CKD subjects and showed a positive correlation with plasma intact parathyroid hormone. Pentosidine was remarkably increased in dialysis patients and inversely correlated with bone-formation rate/bone volume and mineral apposition rate. This study suggests that AGE collagen cross-links strongly associate with disorders of bone metabolism in dialysis patients.

Keywords Collagen cross-link · Advanced glycation end products · Dialysis patient · Mineralization · Hyperparathyroidism

Bone disorders are common complications in chronic kidney disease (CKD) patients. Recently, renal osteodystrophy (ROD) has been redefined as CKD-mineral and bone disorder (CKD-MBD) [1]. The disorders of bone and mineral metabolism associated with CKD-MBD lead to a significantly increased fracture risk [2]. Deterioration in bone calcification and subsequent ectopic calcification such as vascular calcification are common complications. Furthermore, in dialysis patients, bone fragility and increased fracture risk affect quality of life and prognosis [3].

Collagen enzymatic and nonenzymatic cross-links in bone affect not only the mineralization process but also bone material properties [4–7]. In fact, impaired enzymatic cross-linking and/or an increase in nonenzymatic cross-links in bone collagen have been proposed to be determinants of impaired bone quality in aging, osteoporosis, and diabetes mellitus [8]. However, little is known about collagen cross-link formation and its relationship with bone mineralization in CKD patients. Collagen cross-links can be classified into enzymatic types, such as lysyl oxidase (LOX)-mediated cross-links, and nonenzymatic types, such as advanced glycation end products (AGEs) according to mechanism of formation and functional differences [4, 8]. Generally, enzymatic cross-link formation is affected by

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LOX activity and tissue maturation [4], whereas accumulation of nonenzymatic cross-links depends on the degree of glycation and/or oxidation [5, 7, 9]. Although pentosidine is now well established as an AGE, which is formed by glycosylation and oxidation and accumulates markedly in the skin and serum from patients with CKD [5, 10, 11], there are no available data regarding enzymatic and AGE types of cross-link formation in bone collagen.

The aim of this study was to clarify collagen enzymatic and nonenzymatic cross-link formation and the relationship between cross-link formation and the bone-mineralization process in dialysis patients with secondary hyperparathyroidism (2HPT).

Materials and Methods

Patients

Twenty-two patients with severe 2HPT that was resistant to vitamin D therapy participated in this study. Their plasma intact parathyroid hormone (iPTH) concentration persisted at extremely high levels at a similar degree for at least 6 months. They were undergoing regular dialysis therapy; 21 patients were on standard hemodialysis and one was on continuous ambulatory peritoneal dialysis. They consisted of 15 males and seven females; mean age was 56 ± 9 years (range 35–72), and mean dialysis duration was 139 ± 81 months (range 3–357). We performed transiliac bone biopsies, immediately after parathyroidectomy (PTx) under general anesthesia. None of the patients had a history of fracture, were currently using corticosteroids, or were receiving estrogen-replacement therapy. Primary kidney disease diagnoses included nephrosclerosis due to hypertension (two patients), chronic glomerulonephritis (12 patients), diabetic nephropathy (four patients), and other causes (four patients). Blood samples for the determination of biochemical parameters were obtained after an overnight fast. All patients underwent a hemodialysis session the day before PTx.

Bone Biopsy and Bone Histomorphometry

All patients were submitted to a transiliac bone biopsy to evaluate bone histomorphometry and to measure bone collagen cross-links. Of these patients, ten were administered a course of double-labeling tetracycline using demethylchlortetracycline hydrochloride: 300 mg/day for 2 days, with an interval of 5–7 days between treatments. The biopsy was performed 2–7 days after the last dose of tetracycline by the same operator. Undecalcified bone specimens were submitted to standard processing for histological studies at Niigata Bone Science Institute (Niigata Prefecture, Japan). We evaluated cancellous bone histomorphometry of bone volume/tissue volume (BV/TV), osteoid volume/bone volume (OV/BV), fibrosis volume/tissue volume (Fb.V/TV), bone-formation rate/bone volume (BFR/BV), and mineral apposition rate (MAR).

Characterization of Enzymatic and Nonenzymatic Collagen Cross-Links

The reduction of collagen in bone with sodium borohydride ($NaBH_4$; Sigma-Aldrich, St. Louis, MO) and measurement of cross-links were carried out as previously described [12]. Briefly, each bone powder was demineralized twice with 0.5 M EDTA in 50 mM Tris buffer, pH 7.4, for 96 h at 4°C. Demineralized bone residues were then suspended in 0.15 M potassium phosphate buffer, pH 7.6, and reduced at 37°C with $NaBH_4$. Reduced specimens were hydrolyzed in 6 N hydrochloric acid at 110°C for 24 h. Hydrolysates were then analyzed for cross-links on a Shimadzu LC9 HPLC fitted with a cation exchange column (0.9 × 10 cm, Aa pack-Na; Jasco, Tokyo, Japan) linked to an online fluorescence flow monitor (RF10AXL; Shimadzu, Shizuoka, Japan). We determined the content of enzymatic immature reducible and mature nonreducible cross-links. In addition, we determined that pentosidine was one of the well-characterized AGE type of cross-links to evaluate nonenzymatic glycation-induced cross-links. LOX-mediated reducible immature cross-links, dehydro-dihydroxylysinonorleucine (deH-DHLNL), dehydro-hydroxylysinonorleucine (deH-HLNL), and dehydro-lysinonorleucine (deH-LNL) were identified and quantified according to their reduced forms (DHLNL, HLNL, and LNL, respectively). Immature cross-links, such as deH-DHLNL and deH-HLNL, are unstable Schiff bases. In fact, deH-DHLNL and deH-HLNL are present mainly as more stable keto forms such as hydroxylysino-5-keto-norleucine (HLKLN) and lysino-5-keto-norleucine (LKNL), respectively. Our established high-performance liquid chromatography (HPLC) system enables us to determine enzymatic and nonenzymatic cross-link contents within a linear range of 0.2–600 pmol in bone specimens. Enzymatic nonreducible mature cross-links, such as pyridinoline (Pyr) and deoxypyridinoline (Dpyr), and pentosidine were detected by natural fluorescence. The contents of each cross-link were expressed as mole/mole of collagen. In this study, we confirmed that hydrolysates of demineralized and reduced bone specimens consisted of glycine in the range of 322–310 residues per 1,000 amino acids and more than 80 residues of hydroxyproline per 1,000 amino acids, which is in accordance with the known amino acid composition of type I collagen.

We compared the cross-link data from CKD patients to our previously reported Japanese age-matched cadaveric bone study from ilium (mean age 58 ± 8 years, range

40–69, male, $n = 17$, control group) without CKD, diabetes, metabolic disorders, and medications such as vitamin D, vitamin K, and bisphosphonate [8, 12, 13]. Bone specimens of control subjects were also used in our previous study into age-related changes in the biochemical characteristics of collagen from human subjects [13], approved by an independent ethics committee with consent obtained from relatives. Furthermore, the relationship between pentosidine and bone mineralization and the influence of iPTH concentration on bone cross-links in dialysis patients were studied.

Informed Consent

Informed consent to use blood samples, bone specimens, and clinical data for research was given at the time of admission for surgery by the patients themselves. The local official scientific and ethical committee on medical research and the hospital director approved the study.

Statistical Analysis

All values are listed as means with their standard deviation (SD) in the text and tables. For comparison of cross-link parameters between the dialysis patient group and the control group, Student's *t*-test or a Wilcoxon rank sum test was used, as appropriate. Correlations between cross-link parameters with iPTH, BFR/BV, and MAR were analyzed by univariate analysis. All P values were defined as significant at $P < 0.05$. Statistical analyses were performed using JMP, version 5.1.1 for Windows (SAS Institute, Cary, NC).

Results

Patient Characteristics

The clinical characteristics of the dialysis patients are shown in Table 1. All patients had bone and mineral metabolism disorders due to severe 2HPT, regardless of the consequence of conservative therapy such as dietary phosphate limitation, administration of oral phosphate binders, and active vitamin D analogues. None of the patients had been administered calcimimetics. Serum calcium, phosphate, and β_2-microglobulin concentrations were permissive as maintenance dialysis patients. Meanwhile, iPTH and bone alkaline phosphatase (BAP) reflected the severity of increased bone turnover at the time of PTx. Mean values of bone mineral density (BMD) in the total body and the young adult mean (YAM) were 1.023 ± 0.102 g/cm^2 and $88 \pm 9\%$ (range 63–101%), respectively.

Bone Histomorphometry

The results of bone histomorphometry in 22 patients obtained by transiliac bone biopsy are shown in Table 2. According to the classification of ROD [1, 14], 14 patients were mildly affected and eight patients had osteitis fibrosa (OF). None of them had adynamic bone disease. In addition, we evaluated BFR/BV and MAR in ten patients. The mean values of BFR/BV and MAR also indicated high-turnover bone.

Comparison of Bone Collagen Cross-Links

Comparisons of bone collagen cross-links between dialysis patients and the control group are shown in Fig. 1. There was no significant difference in total immature cross-links (the sum of DHLNL, HLNL, and LNL) (Fig. 1a), while total mature pyridinium cross-links (the sum of Pyr and Dpyr) significantly decreased in dialysis patients (Fig. 1b). The total amount of immature and mature pyridinium cross-links showed no difference between the dialysis and control groups (Fig. 1c). The relative ratio of immature cross-links to mature cross-links was significantly higher in dialysis patients (dialysis vs. control 3.41 ± 1.41 vs. 2.62 ± 0.67, $P = 0.048$). Pentosidine content in bone was significantly higher than that in the control group (Fig. 1d).

Correlation Between Bone Collagen Cross-Links and Plasma iPTH Concentration

We studied the correlation between bone collagen cross-links and plasma iPTH concentrations using univariate analysis (Fig. 2). Plasma iPTH concentration significantly and positively correlated with immature cross-links and total amount of immature and mature pyridinium cross-links, whereas neither mature pyridinium cross-links nor pentosidine correlated with iPTH concentration. The relative ratio of immature cross-links to mature cross-links also significantly and positively correlated with plasma iPTH concentration ($Y = 1.942 + 0.002 \times X$; $R^2 = 0.214$, $P = 0.03$).

Correlation Between Pentosidine and Bone Mineralization

Figure 3 shows the correlation between pentosidine and histomorphometric parameters regarding bone mineralization using univariate analysis. Both BFR/BV and MAR negatively correlated with pentosidine.

Discussion

In this study, we evaluated bone collagen cross-links and bone histomorphometry in a total of 22 dialysis patients

Table 1 Patient characteristics

	Dialysis patients ($n = 22$)	Range
Age (years)	56 ± 9	(35–72)
Sex (male/female)	15/7	
Duration of dialysis (months)	139 ± 81	(3–357)
Dialysis modality (HD/CAPD)	21/1	
Diabetes mellitus	4	
Serum calcium (mg/dl)	10.7 ± 0.6	(9.5–11.7)
Serum phosphate (mg/dl)	4.7 ± 1.1	(2.7–6.6)
Serum β_2-microglobulin (mg/l)	25.3 ± 4.3	(13.0–30.2)
Intact PTH (pg/ml)	758 ± 401	(288–2,240)
BAP (U/l)	47.9 ± 23.3	(21.8–100.0)
Total BMD (g/cm^2)	1.023 ± 0.102	(0.842–1.190)

Data are expressed as mean ± SD (range) or number

HD hemodialysis, *CAPD* continuous ambulatory peritoneal dialysis, *PTH* parathyroid hormone, *BAP* bone alkaline phosphatase, *BMD* bone mineral density

Table 2 Results of bone histomorphometry

	Dialysis patients ($n = 22$)
BV/TV (%)	21.89 ± 5.48
OV/BV (%)	5.50 ± 2.28
Fb.V/TV (%)	0.71 ± 0.93
BFR/BV (%/year)[a]	66.13 ± 46.91
MAR (μm/day)[a]	1.011 ± 0.225
Mild type/osteitis fibrosa type	14/8

Data are expressed as mean ± SD or number

BV/TV bone volume/tissue volume, *OV/BV* osteoid volume/bone volume, *Fb.V/TV*, fibrobrast volume/tissue volume, *BFR/BV* bone-formation rate/bone volume, *MAR* mineral apposition rate

[a] $n = 10$

with severe 2HPT. We then compared the actual amount of enzymatic immature and mature cross-links, nonenzymatic cross-links, and pentosidine with those from non-CKD age-matched subjects from our previous study [8, 12, 13]. Generally, the enzymatic cross-linking mechanism in fibrillar collagens is based upon aldehyde formation from specific telopeptide lysine and hydroxylysine residues. These residues are oxidatively deaminated by LOX [4, 8]. It has been shown that hyperhomocysteinemia, observed in CKD patients, inhibits LOX effects and downregulates LOX gene expression [15, 16]. At first, we speculated that immature bone cross-links would be decreased in dialysis patients as increased glycosylation and oxidation of lysine residues due to uremic status could result in inhibiting LOX and reducing enzymatic cross-link formation. According to our HPLC measurements, however, dialysis patients with severe 2HPT did not contribute to reducing enzymatic cross-link formation (Fig. 1c) and immature cross-links had no difference compared with non-CKD subjects (Fig. 1a). Interestingly, plasma iPTH levels positively correlated with total amount of enzymatic cross-links (Fig. 2).

The influence of high levels of plasma iPTH on bone formation has been previously reported [2, 3, 17]. Predominant hyperparathyroid bone disease was associated with a low prevalence of osteoporosis, suggesting an eventual protective effect of PTH on bone [2, 17], which affects rate of turnover. Similar results were also observed in ovariectomized and 5/6 nephrectomized rats with 2HPT, which showed increased BV/TV compared with ovariectomized rats with normal kidney function [18]. Kostenuik et al. [19] previously reported that PTH has additive effects on bone density and mechanical strength in osteopenic ovariectomized rats. Recently, a relationship between human PTH and enzymatic collagen cross-links was reported. Intermittent human PTH administration in ovariectomized cynomolgus monkeys stimulated bone formation, and enzymatic collagen cross-link formation coincided with an increase in bone strength [20]. Paschalis et al. [21] demonstrated that human PTH (teriparatide) stimulated new bone matrix and bone-turnover rate, resulting in a significant decrease in the relative ratio of mature pyridinium to immature cross-link DHLNL. In addition, it has been reported that PTH increased LOX gene expression in bone [22]. Therefore, it seems that high levels of PTH could increase formation of immature collagenous matrix. These findings support our results that dialysis patients may receive a positive effect regarding immature collagen cross-link formation by very high levels of iPTH.

In contrast, enzymatic mature pyridinium cross-link formation was significantly decreased in dialysis patients (Fig. 1b). It is generally thought that the conversion of immature collagen cross-links to mature forms occurs via a

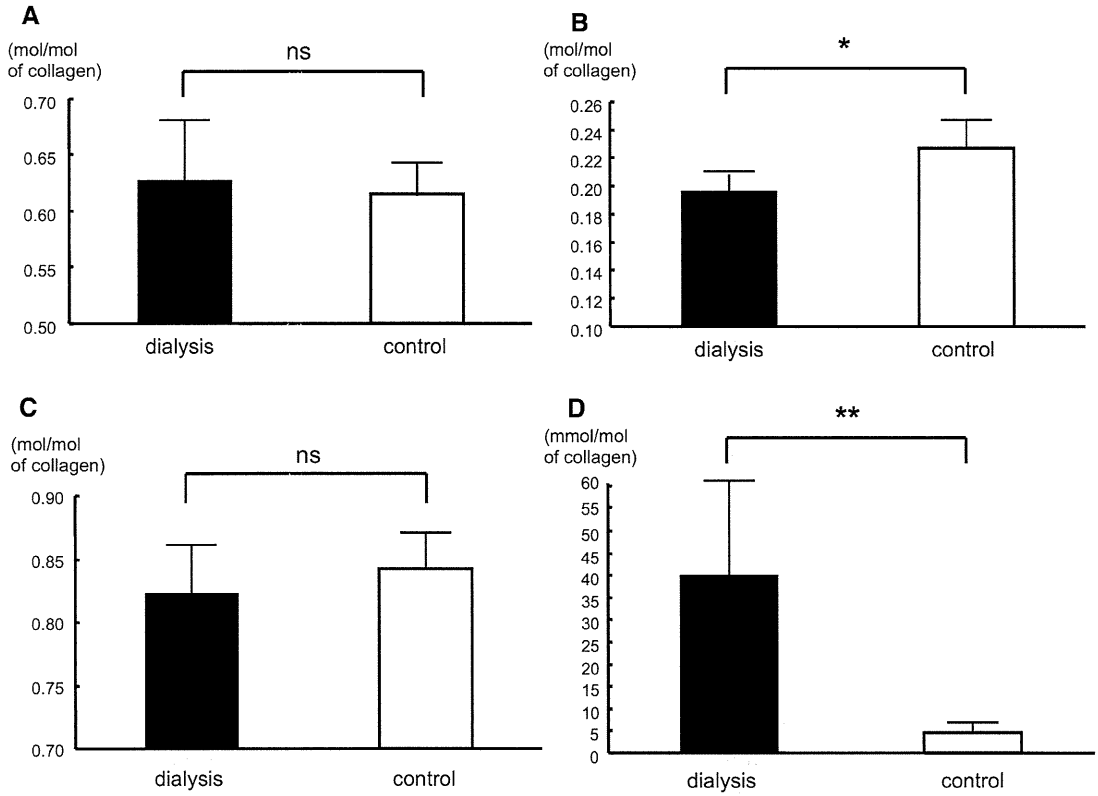

Fig. 1 Comparison of bone collagen cross-links ($n = 22$). **a** Comparison of immature cross-links. **b** Comparison of mature cross-links. **c** Comparison of total amount of immature and mature cross-links. **d** Comparison of pentosidine (AGE collagen cross-links). $*P < 0.01$; $**P < 0.0001$; *ns* not significant. There was no difference in total immature cross-links (**a**), while total mature pyridinium cross-links significantly decreased in dialysis patients ($P < 0.01$, **b**). The total amount of immature and mature pyridinium cross-links showed no difference between the dialysis and control groups (**c**). Pentosidine content in bone was significantly higher than that in the control group ($P < 0.0001$, **d**)

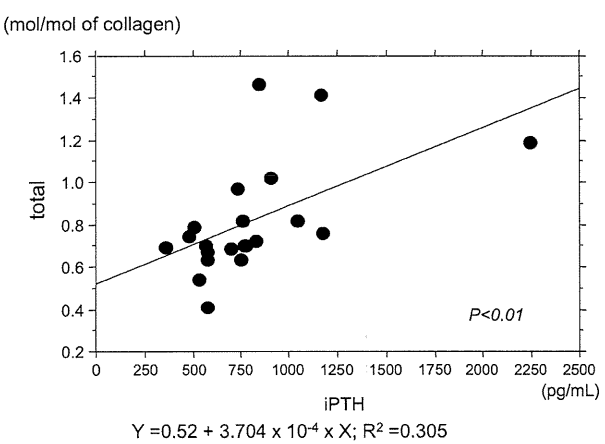

Fig. 2 Correlation between bone collagen cross-links and plasma iPTH concentration ($n = 22$). Plasma iPTH concentration positively correlated with total number of immature and mature pyridinium cross-links ($P < 0.01$)

spontaneous reaction in a time-dependent manner [23, 24]. In a past study of spontaneously diabetic WBN/Kob rats which had low turnover bone, mature pyridinium cross-link formation was maintained compared with the control group, whereas immature cross-link formation was significantly decreased [25]. It was speculated that the prolonged life span of collagen in low-turnover bone could contribute to a time-dependent conversion of immature cross-links to mature forms [25]. As indicated by bone histomorphometric results, none of our subjects appeared to have enough time to consummate cross-link maturation because of the extremely shortened tissue life span induced by increased bone turnover. In addition, we confirmed that the relative ratio of immature cross-links to mature cross-links was significantly higher in dialysis patients (dialysis vs. control 3.41 ± 1.41 vs. 2.62 ± 0.67, $P = 0.048$) and positively correlated with plasma iPTH concentration ($Y = 1.942 + 0.002 \times X$; $R^2 = 0.214$, $P = 0.03$). These findings also suggest that increased plasma iPTH would lead to increased immature and lower mature cross-links through shortened tissue maturation and a positive effect on LOX. Accordingly, we speculate that high bone turnover due to severe 2HPT may contribute to a reduction in enzymatic mature pyridinium cross-link formation

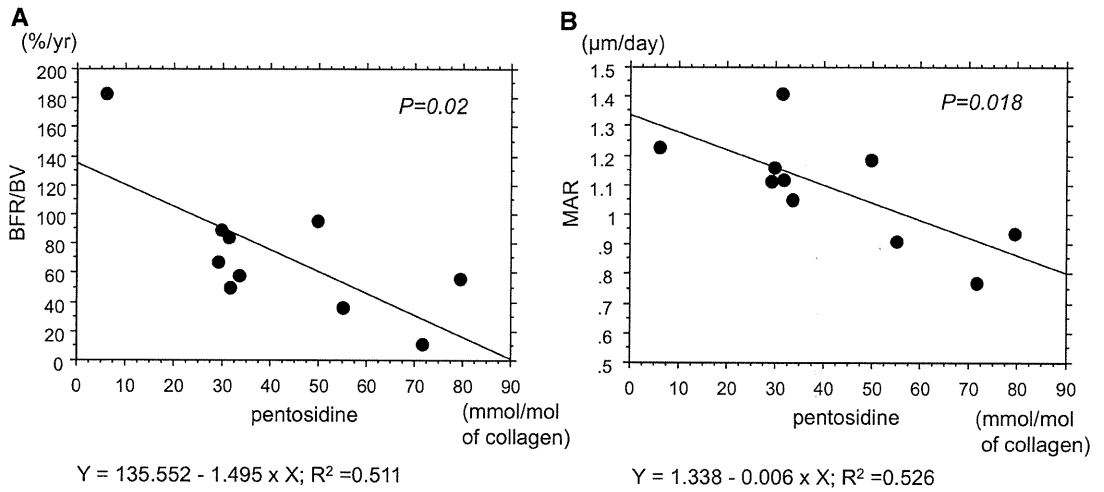

Fig. 3 Correlation between pentosidine content in collagen cross-links and histomorphometric parameters regarding bone mineralization ($n = 10$). **a** Correlation between pentosidine and BFR/BV. **b** Correlation between pentosidine and MAR. Both BFR/BV and MAR negatively correlated with pentosidine (BFR/BV $P = 0.02$, MAR $P = 0.018$)

following a shortened maturing time from immature to mature forms and a shortened life span of bone collagen. This speculation only refers to severe 2HPT patients. Therefore, to investigate our hypothesis, we need additional bone biopsies at several time points if possible and to analyze samples from nonsevere 2HPT patients. However, increased bone turnover may affect enzymatic collagen cross-link maturation and may be an important aspect of bone quality in dialysis patients, as well as bone density.

Pentosidine is now well established as an AGE type of cross-link, which is formed by glycation or oxidation and accumulates in aging, degenerative disease, and diabetic patients with sustained hyperglycemia [26]. Moreover, pentosidine levels markedly increase in tissues and plasma of CKD patients [27]. Carbohydrate-derived AGEs constitute a heterogeneous class of structures, such as pentosidine, which is formed by enzymatic glycation and oxidation reactions between carbohydrate-derived carbonyl compounds and protein amino groups (Maillard reaction). It has been speculated that AGEs and carbonyl stress contribute to complications of long-term dialysis such as cardiovascular disease. Elevated plasma pentosidine content in end-stage kidney disease patients is significantly associated with both inflammation and malnutrition. Low residual renal function and high age further contribute to increased plasma pentosidine concentrations. In uremic patients, circulating pentosidine accumulates as both a protein-linked and a free form. The kidney is very important for excreting free pentosidine [28]. Early glycation products are reversible and do not accumulate in most proteins. In long-lived proteins such as collagen, they may undergo a series of reactions that result in more persistent AGEs or Maillard products. In nonmineralizing tissues such as skin, tendon, and basement membrane, this results in a significant increase in the stiffness of the tissue. In bone, however, which is turned over relatively quickly, AGEs do not accumulate to the same extent. Pentosidine, the only characterized nonenzymatic cross-link in bone collagen, is present at only millimolar levels and has little biomechanical importance in comparison with enzymatic cross-links in normal bone [8]. There are few reports about the role of AGEs in bone mineralization and quality. It was reported that excess AGEs accumulate in adynamic bone disease with diabetes mellitus and elderly patients. According to a laboratory animal study, there was a steady decrease in enzymatic cross-links and a steep increase in pentosidine in spontaneously diabetic WBN/Kob rats after the onset of diabetes [25]. Furthermore, impaired bone mechanical properties in WBN/Kob rats, despite the lack of reduction in BMD, coincided with impaired enzymatic cross-link formation and increases in glycation-induced pentosidine [25]. Meanwhile, it was elucidated that older diabetic women without osteoporosis have an increased risk of fractures associated with decreased bone quality [29]. Yamamoto et al. [30] reported that AGEs are involved in the pathogenesis of adynamic bone disease by inhibiting osteoblastic activity and by inhibiting PTH secretion in response to hypocalcemia.

In this study, pentosidine increased approximately 10-fold in dialysis patients compared with non-CKD subjects. This finding suggested that uremic status and high levels of oxidative stress in dialysis patients were followed by significant AGE collagen cross-link formation. Moreover, very high levels of AGEs in bone collagen cross-links may have potentially adverse effects on bone formation and mineralization, whereas all of our subjects had severe

2HPT, which is crucial in the determination of bone formation and mineralization. However, there was no significant correlation between plasma iPTH levels and pentosidine levels in collagen cross-links.

We examined BFR/BV and MAR with double-labeling tetracycline in 10 dialysis patients to determine correlations between bone collagen cross-links and bone formation and mineralization. Cross-sectional analysis of transiliac bone biopsies showed that BFR/BV and MAR negatively correlated with pentosidine (BFR/BV $r^2 = 0.511$, $P = 0.02$; MAR $r^2 = 0.526$, $P = 0.018$). These data suggest that AGEs could have an important role in bone formation and the mineralization process. Recently, Sanguineti et al. [31] reported that pentosidine inhibited differentiation and proliferation of human osteoblastic cells in vitro. This provides further support for the detrimental effect of AGEs on bone that leads to functional alterations of osteoblasts [32]. Very high levels of AGEs, as observed in dialysis patients, may contribute to inhibiting both differentiation of osteoblastic cells and subsequent mineralization of collagen cross-links. Although plasma iPTH levels positively correlated with BFR/BV ($P < 0.001$, data not shown) and indicated high turnover of bone, it was speculated that bone formation and mineralization could be relatively impaired by increased AGEs. Generally, aging, glycation, and oxidation lead to AGE formation. Bone collagen has a long life, making it susceptible to glycation and oxidation. The generation and accumulation of AGEs in bone tissue could therefore contribute to the deterioration of bone quality. In postmenopausal osteoporotic patients with hip fractures, AGE cross-link content of low-mineralized, newly formed bone fraction was significantly higher than that of high-mineralized, previously formed bone fraction [6]. Moreover, AGE cross-link content was significantly higher in both low-mineralized and high-mineralized bone fractions from fracture cases compared with nonfracture cases [6]. These data indicate that even if healthy collagen cross-links are newly formed by bone turnover, subsequent collagen maturation could be inhibited by harmful environmental factors such as uremic status with very high levels of AGEs regardless of duration and past severity of disease. Accordingly, this could result in deteriorated bone quality. However, our data did not reveal the cause of disorders in bone mineralization or whether it was dependent on the duration or amplitude of AGE cross-link formation. In this study, we did not evaluate dialysis patients with mild to moderate 2HPT. Further studies are therefore required.

There are limitations to the interpretation of the results of this study. First, enzymatic mature pyrrole cross-links may be equally important as pyridinium cross-links [8]. Pyrrole cross-links are unstable during acid hydrolysis, and therefore, we cannot measure them using our HPLC method. Since the major determinant of the total amount of immature lysinonorleucine type and their mature forms, such as pyridinium and pyrrole, is LOX activity, overall formation of pyrrole cross-links might be similar to other LOX-controlled cross-linking. Second, pentosidine is just one of many AGEs in bone. The reasons we elected to quantify pentosidine are that pentosidine can be quantified easily and precisely in small (<1 mg) bone specimens [12]. Recently, Tang and Vashishth [33, 34] reported a positive correlation between pentosidine and bulk fluorescent AGEs ($r^2 = 0.318$), suggesting that the pathway of their formation may be similar and that pentosidine may be used as a biomarker of AGEs. Furthermore, glucosepane is a major type of AGE cross-link that accumulates in the human extracellular matrix with aging in the skin and glomerular basement membrane [35]. In this study, unfortunately, we obtained just a small amount of bone powder (<2 mg) for biochemical analysis. Thus, we analyzed conventional immature divalent cross-links, mature pyridinium cross-links, pentosidine, and hydroxyproline analysis after acid hydrolysis for singe-column HPLC. To determine the content of glucosepane, a different type of enzymatic digestion after demineralization is needed. Two milligrams of bone powder reduced significantly its mass and weight due to removal of the mineral phase (about <1 mg). Such a small amount of bone specimen could not determine exactly the content in bone by duplicate or triplicate analyses. Glucosepane has been reported to be the major nonfluorescent glycation cross-link, and its concentration can be almost equivalent to the enzymatic cross-links, that is, one or two per collagen molecule. In contrast, the pentosidine concentration is one cross-link per several hundred collagen molecules and, therefore, can have little effect on the mechanical properties of the fiber in comparison to glucosepane. Glucosepane, the most prominent nonfluorescent AGE cross-link, is an acid-labile, lysine-argine-derived cross-link as well as pentosidine. Biemel et al. [36] demonstrated the structural similarities between pentosidine and glucosepane, thus suggesting a parallel mechanism in the respective formation pathways. Because glucosepane is formed in skin collagen with aging and is present in sufficient quantities to affect the physical properties of skin collagen fiber, type I collagen may form in bone and thereby affect the material properties. To date, though, glucosepane formation in bone and age-related change have not been reported. Thus, we should confirm that glucosepane is formed in bone collagen as well as skin and basement membrane, and we should attempt to clarify the role of glucosepane in human bone.

In conclusion, glycation-induced nonenzymatic cross-linking pentosidine significantly increases in bone collagen and potentially detrimentally affects bone formation and mineralization in dialysis patients with severe 2HPT. This study suggests that very high levels of AGE collagen

cross-links are strongly associated with disorders of bone metabolism in dialysis patients.

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AJKD
Original Investigation

Cost-Effectiveness of Cinacalcet Hydrochloride for Hemodialysis Patients With Severe Secondary Hyperparathyroidism in Japan

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Background: Cinacalcet effectively reduces elevated levels of parathyroid hormone (PTH) in patients with secondary hyperparathyroidism (SHPT), even those with severe disease for whom parathyroidectomy can be the treatment of choice. The objective of this study was to estimate the cost-effectiveness of cinacalcet treatment in hemodialysis patients with severe SHPT in Japan.

Study Design: Cost-effectiveness analysis.

Setting & Population: Patients with severe SHPT (intact PTH >500 pg/mL) who were receiving hemodialysis in Japan.

Model, Perspective, & Timeframe: A Markov model was constructed from the health care system perspective in Japan. Patients were followed up over their lifetime. Dialysis costs were not included in the base case.

Intervention: Cinacalcet as an addition to conventional treatment compared to conventional treatment alone. In both arms, patients underwent parathyroidectomy if intact PTH level was >500 pg/mL for 6 months and they were eligible for surgery.

Outcomes: Costs, quality-adjusted life-years (QALYs), and incremental cost-effectiveness ratios (ICERs).

Results: ICERs for cinacalcet for those who were eligible for surgery and those who were not were $352,631/QALY gained and $21,613/QALY gained, respectively. Sensitivity and scenario analyses showed that results were fairly robust to variations in model parameters and assumptions. In the probabilistic sensitivity analysis, cinacalcet was cost-effective in only 0.9% of simulations for those eligible for surgery, but in more than 99.9% of simulations for those ineligible for surgery, if society would be willing to pay $50,000 per additional QALY.

Limitations: Data for the long-term effect of cinacalcet on patient-level outcomes are limited. The model predicted rates for clinical events using data for the surrogate biochemical end points.

Conclusions: The use of cinacalcet to treat severe SHPT is likely to be cost-effective for only those who cannot undergo parathyroid surgery for medical or personal reasons.

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INDEX WORDS: Cinacalcet hydrochloride; cost-effectiveness; hemodialysis; secondary hyperparathyroidism.

With the growing number of patients with end-stage renal disease, the demand for dialysis therapy places a heavy financial burden on health care payers, exceeding $23 billion in the United States[1] and $13 billion in Japan.[2] Secondary hyperparathyroidism (SHPT), a common complication of dialysis patients,[3,4] is an important subject for economic analysis because treatment for this disease and its related complications, such as cardiovascular (CV) disease and bone fracture, can result in increased expenditure.[5] Although the most commonly recognized complication of SHPT is renal bone disease,[6] recent observational studies indicate that elevations in biochemical parameters of SHPT are associated with increased mortality and CV morbidity.[7-13] Severe SHPT can also decrease quality of life by causing symptoms of bone pain, muscle weakness, and itching.[14-16]

Conventional treatment for SHPT includes the administration of phosphate binders and active vita-

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min D derivatives. However, a significant proportion of patients are refractory to these treatments, particularly for those with severe disease. Parathyroidectomy is the definitive therapy for treating such uncontrolled SHPT.[14-19] Cinacalcet hydrochloride is the latest treatment option for therapeutic control of SHPT. Treatment with cinacalcet effectively decreases parathyroid hormone (PTH) levels in patients with uncontrolled SHPT while adequately maintaining acceptable levels of calcium and phosphorus.[20-25]

However, controversy remains about whether administration of cinacalcet is a cost-effective approach for the management of severe SHPT. Because surgical costs for parathyroidectomy usually occur only once in a lifetime, it is presumed that parathyroidectomy is more cost-effective than permanent treatment with cinacalcet.[26] However, there are certain patients who cannot undergo surgery for medical or personal reasons. Therefore, in this study, we separately estimated the cost-effectiveness of cinacalcet to treat severe SHPT for those who were eligible for parathyroidectomy and those who were not. The analyses presented here are specific to the Japanese setting; however, results were robust to various sensitivity analyses and would have important implications for the management of SHPT worldwide.

METHODS

Study Design

We constructed a Markov model to estimate quality-adjusted life-years (QALYs) and lifetime costs associated with cinacalcet plus conventional treatment compared with conventional treatment alone for the treatment of severe SHPT. Because no randomized trials have evaluated whether treatment of SHPT with cinacalcet reduces the risk of mortality and CV morbidity, we modeled the effect of cinacalcet on patient-level outcomes using data from observational studies on the risk of clinical events in relation to biochemical parameters of SHPT. The incremental cost-effectiveness ratio (ICER) was calculated using the following formula: $ICER = (Cost_{cinacalcet+std} - Cost_{std})/(QALY_{cinacalcet+std} - QALY_{std})$, where std refers to conventional treatment. An annual discount rate of 3% was applied to both costs and health benefits. All analyses were performed using TreeAge Pro 2009 (TreeAge Software, www.treeage.com). Ethics approval was not required for this project.

Population

The modeled population was Japanese hemodialysis patients with severe SHPT, defined as intact PTH level >500 pg/mL. A hypothetical cohort of 1,000 patients was modeled until the entire cohort died. The starting age for the cohort was 55 years, based on the mean age of participants in clinical trials of cinacalcet in Japan.[22-24] Analyses were performed for 2 types of cohorts separately: (1) those who were eligible for parathyroidectomy and (2) those who were ineligible for parathyroidectomy for medical or personal reasons.

Model Structure

The model diagram is shown in Fig 1. After initial treatment with either conventional treatment alone or conventional treatment

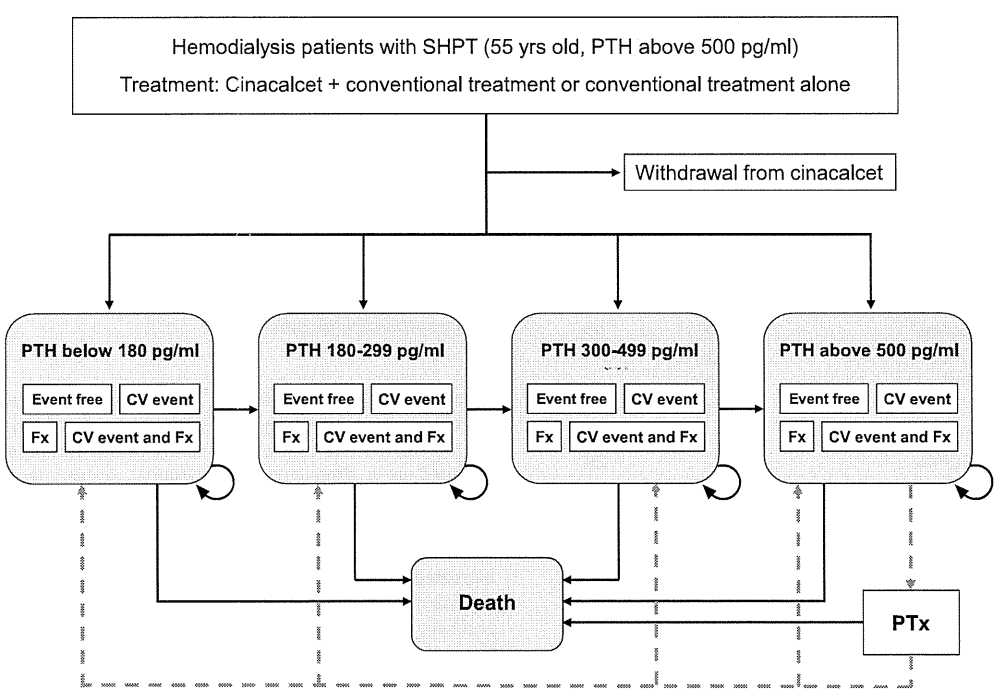

Figure 1. Model diagram. Abbreviations: CV, cardiovascular; Fx, fracture; PTH, parathyroid hormone; PTx, parathyroidectomy; SHPT, secondary hyperparathyroidism.

plus cinacalcet, patients were divided into 4 PTH categories (<180, 180-300, 301-500, and >500 pg/mL), depending on the treatment received. The cycle length of the model was 3 months. In each cycle, patients experienced one of the following clinical events: event free, CV event, fracture, or CV event and fracture. For patients who were eligible for surgery, parathyroidectomy occurred when they had severe SHPT (intact PTH >500 pg/mL) for 6 months irrespective of the treatment received. Patients who underwent successful parathyroidectomy were assumed to gain improved control of PTH levels (intact PTH <180 pg/mL). Because SHPT is a progressive disease, patients in the conventional treatment arm were assumed to move to higher PTH categories over time. Patients who withdrew from cinacalcet treatment due to adverse effects received conventional treatment, with transition probabilities similar to those in the conventional treatment arm. Costs and benefits for these patients continued to be counted within the cinacalcet arm.

Transition Probabilities

The initial transition probabilities to 4 PTH categories in both treatment arms were estimated using patient-level data derived from a clinical trial of cinacalcet conducted in Japan[22] (Table 1). The proportion of withdrawal from cinacalcet was obtained from this trial[22] and another dose-finding study.[23] Transition to higher PTH categories was assumed to occur at a rate of 10% per year in the conventional treatment arm. This assumption was not applied to patients treated with cinacalcet based on the report that cinacalcet can effectively exert long-term control of PTH levels without dose escalation.[27]

A transition probability (P) of an event occurring over a time interval (t) was calculated using a rate (r) according to the following formula: $P = 1 - \exp(-rt)$.[28] Table 2 lists the input parameters used to calculate the probabilities of clinical outcomes. The incidence of non-CV death and relative risk by PTH category were derived from a nationwide registry of Japanese dialysis patients.[12,29] The incidence of CV events was derived from the Q Cohort Study, a large-scale prospective observational study of 3,170 hemodialysis patients in Kyushu, Japan.[30] The rate of death after CV events also was derived from the Q Cohort Study.[30] The relative risk of CV events by PTH category was derived from the Japan Dialysis Outcomes and Practice Patterns Study (J-DOPPS).[11] Because cinacalcet decreases not only PTH levels, but also serum calcium and phosphorus levels,[20-25] the potential impact of these effects on risk of mortality and CV morbidity was incorporated in the model. The rate of death due to CV events and other causes is modeled as a time-dependent variable, derived from national registry data of Japanese dialysis patients.[31]

The incidence of fracture and relative risk by PTH category were obtained from the Q Cohort Study.[30] To account for an increased risk of fracture associated with older age and female sex, the risk of fracture by PTH category was multiplied by relative risk according to age and sex group derived from the DOPPS.[32] Because studies have shown inconsistent results for the association between serum PTH level and risk of fracture,[32-34] we performed a sensitivity analysis in which this association was not applied in the model. The relative risk of subsequent

Table 1. Transition Probabilities

Variable	Value	Source
Initial transition probability		
Cinacalcet + conventional treatment		
PTH <180 pg/mL	0.2	22
180-300 pg/mL	0.27	
301-500 pg/mL	0.37	
>500 pg/mL	0.16	
Conventional treatment		
PTH <180 pg/mL	0	22
180-300 pg/mL	0.02	
301-500 pg/mL	0.1	
>500 pg/mL	0.88	
Probability of withdrawing cinacalcet	0.086	22, 23
Annual transition probability to a higher PTH range		
Cinacalcet + conventional treatment	0	Assumption
Conventional treatment	0.1	Assumption

Abbreviation: PTH, parathyroid hormone.

Table 2. Incident Rate and Relative Risk of Clinical Events

Variable	Value	Source
Annual mortality rate due to non-CV causes	0.051	12, 29
RR of non-CV death		
Cinacalcet + conventional treatment		
PTH <180 pg/mL	0.895	12, 22
180-300 pg/mL	1.010	
301-500 pg/mL	1.110	
>500 pg/mL	1.226	
Conventional treatment		
PTH <180 pg/mL	0.959	12, 22
180-300 pg/mL	1.082	
301-500 pg/mL	1.189	
>500 pg/mL	1.314	
Annual incidence rate of CV event	0.130	30
RR of CV event		
Cinacalcet + conventional treatment		
PTH <180 pg/mL	1.011	11, 22
180-300 pg/mL	0.929	
301-500 pg/mL	0.966	
>500 pg/mL	1.224	
Conventional treatment		
PTH <180 pg/mL	1.213	11, 22
180-300 pg/mL	1.116	
301-500 pg/mL	1.160	
>500 pg/mL	1.349	
Probability of death after CV event	0.379	30
RR of death according to age	1.057	31
Annual incidence rate of Fx	0.012	30
RR of Fx with severe SHPT	2.0	30
RR of Fx (men:women)		
55-64 y	0.702:0.604	32
65-74 y	0.777:1.525	
75- y	1.649:2.545	
RR of subsequent Fx	2.1	30
RR of death after Fx	1.7	30
Rate of GI events during cinacalcet treatment	0.17	22, 23

Abbreviations: CV, cardiovascular; Fx, fracture; GI, gastrointestinal; PTH, parathyroid hormone; RR, relative risk; SHPT, secondary hyperparathyroidism.

Table 3. Clinical Parameters for Parathyroidectomy

Variable	Value	Source
Waiting period for parathyroidectomy (mo)	0	Assumption
Probability of death after parathyroidectomy	0.0015	35
Transition probability after parathyroidectomy		
PTH <180 pg/mL	0.958	35
180-300 pg/mL	0.026	
301-500 pg/mL	0.009	
>500 pg/mL	0.008	

Abbreviation: PTH, parathyroid hormone.

fracture and the relative risk of death after fracture were derived from the Q Cohort Study.[30]

Transition probabilities after parathyroidectomy were derived from a study by Tominaga et al,[35] who examined the clinical course of 1,156 patients who underwent parathyroidectomy for severe SHPT (Table 3). The risk of death as a complication of parathyroidectomy also was applied in the model. Patients who underwent successful parathyroidectomy were assumed to not continue receiving cinacalcet after surgery.

Costs

Costing was undertaken from the perspective of the third-party health care payer in Japan (Table 4). Drug costs were obtained from the 2010 National Health Insurance Price List set by the Ministry of Health, Labor, and Welfare in Japan.[36] Costs of cinacalcet per cycle were calculated using the doses of cinacalcet used for patients who had intact PTH levels >500 pg/mL at baseline in the long-term study.[24] Costs for conventional treatment were calculated using data from a baseline analysis of the Mineral and Bone Disorder Outcomes Study for Japanese CKD Stage 5D Patients (MBD-5D).[37] We assumed that costs of active vitamin D and phosphate binders do not change during treatment with cinacalcet, based on data from an interim analysis of the MBD-5D.[38] We explored whether this assumption affects the cost-effectiveness of cinacalcet in the sensitivity analysis. Surgical costs for parathyroidectomy with autotransplant were calculated by a combination of fee for service and a per diem inclusive rate set by the Diagnosis Procedure Combination.[39] Because intravenous active vitamin D treatment usually is discontinued after successful parathyroidectomy, we assumed that costs for medications to treat SHPT substantially decrease after surgery.

Given the lack of data for costs for CV events and fracture for dialysis patients, we used data from other populations. Costs for CV events (angina pectoris, myocardial infarction, and cerebrovascular accident) were derived from an economic analysis of Japanese patients with hypertension,[40] with a weighted average according to frequencies reported in the Q Cohort Study.[30] Costs for fracture were derived from a study of patients with hip fracture in Japan.[41] Dialysis costs were derived from the report by the Japanese Association of Dialysis Physicians,[42] but were not included in the base-case analysis. All costs were calculated in Japanese Yen and converted into US dollars using the Organisation for Economic Co-operation and Development purchasing power parity rate in 2010 ($1 = ¥111).

Utilities

Quality of life was incorporated into the model through the use of utility values, ranging from zero at death to one at perfect health. The utilities used in the analysis are listed in Table 4. The utility value for hemodialysis patients was derived from 36-Item Short Form Health Survey (SF-36) scores of Japanese hemodialysis patients[43,44] using a formula to convert SF-36 scores to Short-Form 6 Dimensions (SF-6D) utility values.[45] To reflect decreased quality of life associated with symptoms of severe SHPT,[14-16] a 15% reduction in the utility value was incorporated into the analysis for patients with severe SHPT, as assumed in the study by Garside et al.[46] Because treatment with cinacalcet occasionally causes mild gastrointestinal symptoms, at a rate 17% higher than the placebo group,[22,23] we incorporated these effects in the analysis. In the absence of condition-specific data, we assumed a scaled reduction of 5% in utility for those who can tolerate cinacalcet, but with mild gastrointestinal symptoms.

Because no studies provided utility values for dialysis patients after CV events or fracture, we used utility data derived from other populations, which were then multiplied by the event-free utility value for hemodialysis patients. Utility values for CV events in both acute and chronic phases were derived from a variety of data sources,[47-49] which were weighted by the reported frequencies of each CV event in the Q Cohort Study.[30] Utility values for fracture were derived from a study of Japanese patients with osteoporosis-related fracture reported by Hagino et al.[50]

Sensitivity Analyses

We performed 1-way sensitivity analyses to examine whether alterations in key input parameters and assumptions affect results of the base-case analysis. To further explore uncertainty in all parameter estimates, we performed a probabilistic sensitivity analysis using Monte Carlo simulations. In each of the 1,000 simula-

Table 4. Costs and Utility Values

Variable	Value	Source
Cost (US$)		
Cinacalcet treatment (/cycle)	874.9 (54 mg/d)	24, 36
Conventional treatment (/cycle)	594.7	36, 37
Conventional treatment after parathyroidectomy (/cycle)	135	Assumption
Parathyroidectomy (/operation)	5,186	39
Treatment for CV event (/event)	13,569	30, 40
Treatment for fracture (/event)	19,892	41
Hemodialysis (/cycle)	10,176	42
Utility		
Event free	0.680	43-45
Severe SHPT	×0.85	Assumption
Mild GI adverse event	×0.95	Assumption
CV event	×0.477	30, 47, 48
Fracture	×0.469	50
CV event and fracture	×0.224	30, 47, 48, 50
Previous CV event	×0.787	49
Previous fracture	×0.855	50
Previous CV event and fracture	×0.673	49, 50

Abbreviations: CV, cardiovascular; GI, gastrointestinal; SHPT, secondary hyperparathyroidism.

Table 5. Clinical Outcomes Predicted in the Base Case

	Conventional Treatment	Cinacalcet + Conventional Treatment
Patients eligible for parathyroidectomy		
CV event (events/100 patient-years)	15.1	14.0
Fracture (events/100 patient-years)	1.1	1.1
Parathyroidectomy (operations/100 patients)	90.1	23.9
Mean survival (y)	11.00	11.00
Patients ineligible for parathyroidectomy		
CV event (events/100 patient-years)	20.8	14.0
Fracture (events/100 patient-years)	1.9	1.5
Mean survival (y)	7.79	10.53

Abbreviations: CV, cardiovascular.

tions, the value for each model input was randomly selected from its distribution (Table S1, available as online supplementary material). We defined types of probability distribution for each variable when data for variability were available (Item S1). A cost-effectiveness acceptability curve was constructed to estimate the proportion of simulations in which the addition of cinacalcet would be preferred in terms of cost-effectiveness assuming willingness-to-pay thresholds of $50,000 and $100,000 per additional QALY.

RESULTS

Clinical Outcomes

Patient-level outcomes in the economic model for 1,000 patients are listed in Table 5. For patients who are eligible for parathyroidectomy, the addition of cinacalcet to conventional treatment resulted in a marked decrease in the incidence of parathyroidectomy, but there were only slight differences in the incidences of CV events, fracture, and mortality between the arms of the model. In contrast, use of cinacalcet for those ineligible for parathyroidectomy was predicted to result in decreased incidences of CV events and fracture and improved survival compared with conventional treatment alone.

Cost-Effectiveness

Base-case results for the incremental cost-effectiveness of cinacalcet are listed in Table 6. For patients who are eligible for parathyroidectomy, cinacalcet treatment conferred a slight increase in quality-adjusted life expectancy (0.079 QALYs), but cost an additional $27,858 per person, resulting in an ICER of $352,631/QALY gained. In contrast, cinacalcet treatment for those ineligible for parathyroidectomy resulted in a significant improvement in clinical outcomes, along with increased lifetime costs. The incremental costs and QALYs were $24,812 and 1.147, respectively, yielding an ICER of $21,613/QALY gained.

One-Way Sensitivity Analysis

The base-case result for those eligible for parathyroidectomy was robust to several 1-way sensitivity analyses. Over the full range of model parameters, ICERs remained higher than $100,000/QALY gained (Fig 2). Even when we modeled a waiting period for parathyroidectomy of 12 months, the ICER remained more than $100,000/QALY gained. Cinacalcet treatment would be preferred if the cost of cinacalcet decreased by 95%, if society would be willing to pay $100,000/QALY gained. For patients who are ineligible for parathyroidectomy, the base-case result was robust to alterations in various key parameters and assumptions (Fig 3). Even when we included the costs of dialysis in the analysis, the ICER for cinacalcet remained $59,986/QALY gained.

Scenario Analysis

In the base-case analysis, we modeled the effects of cinacalcet on serum calcium and phosphorus levels for the risk of clinical events at different levels of PTH control. However, it is still unknown whether these alterations in biochemical parameters additively improve clinical outcomes in patients treated with cinacalcet. We therefore performed a scenario analysis in which

Table 6. Base-Case Cost-Effectiveness Results per Patient for Cinacalcet

	Cost (US$)	QALYs	Incremental Costs	Incremental QALYs	ICER (US$/QALY)
Patients eligible for parathyroidectomy					
Conventional treatment	30,198	5.172			
Cinacalcet + conventional treatment	58,056	5.252	27,858	0.079	352,631
Patients ineligible for parathyroidectomy					
Conventional treatment	38,812	3.825			
Cinacalcet + conventional treatment	63,624	4.973	24,812	1.147	21,613

Abbreviations: ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life-year.

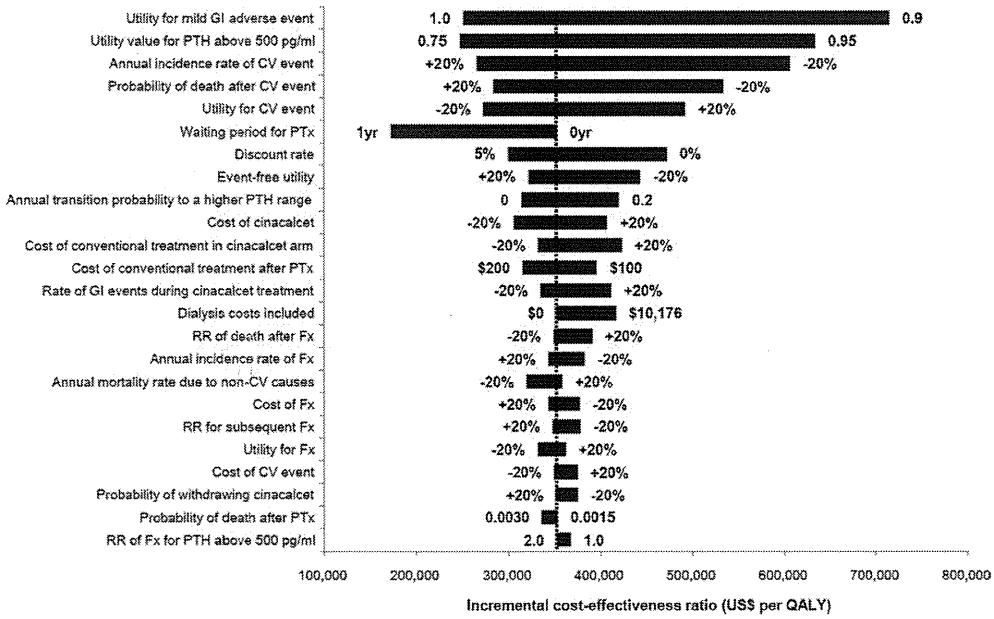

Figure 2. One-way sensitivity analyses on variables that most influenced the incremental cost-effectiveness of cinacalcet for those who were eligible for PTx. Abbreviations: CV, cardiovascular; Fx, fracture; GI, gastrointestinal; PTH, parathyroid hormone; PTx, parathyroidectomy; QALY, quality-adjusted life-year; RR, relative risk.

we did not consider the effects of cinacalcet on serum calcium and phosphorus levels for the risk of clinical events. In this scenario, the addition of cinacalcet for those who were eligible for parathyroidectomy resulted in a slight decrease in QALYs of 0.046, but cost an additional $28,163 per person; thus, cinacalcet was "dominated" by conventional treatment alone. For those who were ineligible for parathyroidectomy, the ICER for cinacalcet remained $29,638/QALY gained (Table S2).

We also performed a scenario analysis in which the modeled population was restricted to those receiving intravenous active vitamin D at baseline, and these agents were assumed to be changed to oral administrations in the cinacalcet arm. In this scenario, the ICER

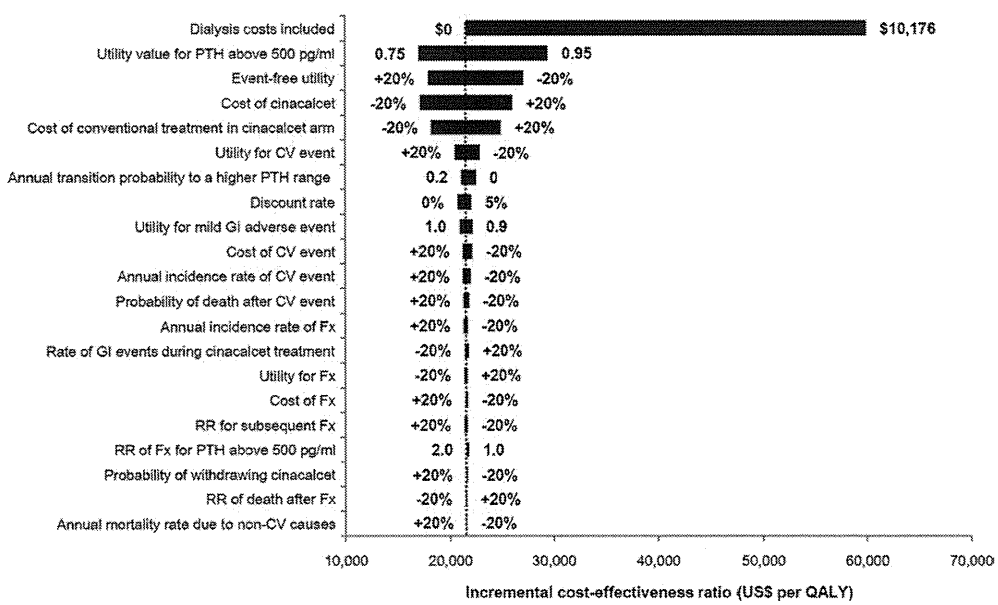

Figure 3. One-way sensitivity analyses on variables that most influenced the incremental cost-effectiveness of cinacalcet for those who were ineligible for parathyroidectomy (PTx). Abbreviations: CV, cardiovascular; Fx, fracture; GI, gastrointestinal; PTH, parathyroid hormone; QALY, quality-adjusted life-year; RR, relative risk.

for those eligible for parathyroidectomy decreased to $259,155/QALY gained, but still was much higher than $100,000 per additional QALY. For those who were ineligible for parathyroidectomy, the ICER for cinacalcet further decreased to $6,038/QALY gained (Table S3).

Finally, we describe an alternate scenario in which the population considered was patients with more severe SHPT (intact PTH >800 pg/mL) and surgical parathyroidectomy was indicated if intact PTH levels were >800 pg/mL for 6 months. In this scenario, ICERs for cinacalcet for those who were eligible for surgery and those who were not were $415,034/QALY gained and $25,024/QALY gained, respectively (Table S4).

Probabilistic Sensitivity Analysis

In the Monte Carlo simulation varying all parameters simultaneously, cinacalcet for those who were eligible for parathyroidectomy was cost-effective in 0.9% of the simulations when assuming a willingness-to-pay threshold of $50,000 per additional QALY and in 4.9% of the simulations when assuming a willingness-to-pay threshold of $100,000 per additional QALY (Fig S1). In contrast, cinacalcet for those who were ineligible for surgery was cost-effective in more than 99.9% of the simulations using a willingness-to-pay threshold of $50,000 or $100,000 per additional QALY (Fig S2).

DISCUSSION

Many clinical trials have shown that cinacalcet effectively controls biochemical parameters of SHPT.[20-25] However, just a few studies to date have estimated the cost-effectiveness of this agent. Narayan et al[26] compared the cost-effectiveness of cinacalcet and parathyroidectomy in patients who were potential candidates for surgery in the United States and showed that long-term use of cinacalcet to treat severe SHPT is unlikely to be cost-effective compared with parathyroidectomy. Another report from the United Kingdom by Garside et al[46] showed that the addition of cinacalcet is unlikely to be cost-effective compared with conventional treatment alone for the treatment of SHPT by analyzing those who were eligible for surgery and those who were not together. However, in the real world, whether the patient can undergo surgery can affect the decision to administer cinacalcet for treating SHPT. There are a certain number of patients who cannot undergo parathyroidectomy for medical or personal reasons: those who cannot tolerate general anesthesia, those who cannot be positioned with cervical extension, those with severe SHPT due to parathyromatosis, or those who refuse surgery.

In this economic analysis, we therefore examined the cost-effectiveness of cinacalcet for those who were eligible for parathyroidectomy and those who were not separately. As expected, our analysis showed that cinacalcet was unlikely to be cost-effective for those eligible for parathyroidectomy, consistent with the study by Narayan et al[26] and the scenario analysis restricted to those eligible for parathyroidectomy in the study by Garside et al.[51] In contrast, for those who cannot undergo parathyroidectomy, our analysis showed that cinacalcet was likely to be a cost-effective option. To the best of our knowledge, this is the first cost-effectiveness analysis of cinacalcet for those ineligible for surgery. Our results may provide an important rationale for the use of cinacalcet for these patients in terms of cost-effectiveness.

The main limitation of our analysis is that the effect of cinacalcet on patient outcomes was estimated from the effect on biochemical variables of SHPT. Because elevations in serum calcium, phosphorus, and PTH levels have been associated with increased morbidity and mortality in observational studies,[7-13] it is theoretically reasonable to assume that these reductions by cinacalcet result in improved clinical outcomes. However, we should emphasize that the effect of therapeutic interventions on the surrogate marker does not always translate into favorable effects on the real outcome.[52,53] Nevertheless, the improved clinical outcomes in the cinacalcet arm predicted in our analysis are consistent with results of a post hoc analysis of randomized trials showing that treatment with cinacalcet resulted in improvement in the risk of parathyroidectomy, fracture, and CV hospitalization and specific components of health-related quality of life[54] and with results of a recent observational study showing a significant survival benefit associated with cinacalcet prescription in the US dialysis population.[55] Currently, the EVOLVE (Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events) trial is underway to determine whether treatment with cinacalcet results in reductions in mortality and CV morbidity.[56] The validity of our simulation model and results should be ascertained in the ongoing EVOLVE trial in the future.

It is important to note that administration of cinacalcet can affect prescribing patterns for concurrent medications. Although recent clinical trials focused on strategies for managing SHPT with cinacalcet in combination with low-dose active vitamin D,[57-59] reductions in serum calcium and phosphorus levels during cinacalcet treatment also may allow clinicians to use vitamin D analogues more actively. Thus, the impact of cinacalcet administration on concurrent medications may vary according to the physician's practice patterns and individual treatment response. We there-

fore explored whether the changes in costs of concurrent medications affect the cost-effectiveness of cinacalcet and found that results were insensitive to these changes. We also performed a scenario analysis in which we assumed that intravenous active vitamin D was changed to oral administration during cinacalcet treatment. Even in this scenario, which is weighed in favor of cinacalcet, treatment with cinacalcet was acceptable for only those who were ineligible for parathyroidectomy, similar to the base-case results.

Our cost-effectiveness analysis was performed from the health care system perspective in Japan, and parameters for the simulation model were derived mostly from Japanese studies. It is important to acknowledge that Japanese dialysis patients are characterized by a lower risk of CV disease and all-cause mortality compared with dialysis populations in other countries.[60] Also noteworthy is that the cost of cinacalcet in Japan is substantially lower than that in other countries. In addition, the Japanese guideline recommends surgical parathyroidectomy for patients with intact PTH levels >500 pg/mL,[61] which may be lower than the threshold for parathyroidectomy in other countries. However, our results were robust to changes in key input parameters, including variables that vary from country to country. Therefore, we believe our results will provide useful information for the cost-effective use of cinacalcet in other countries.

Finally, it should be mentioned that we did not include the costs of dialysis in the base-case analysis. Although still controversial, exclusion of dialysis costs generally is considered adequate in cost-effectiveness analyses because their inclusion could result in refusal to accept interventions that are relatively inexpensive but could improve patient survival.[62] Nevertheless, even when we included the costs of dialysis in the present economic analysis, the ICER for cinacalcet for those ineligible for parathyroidectomy remained $59,986/QALY gained, further supporting the cost-effectiveness of cinacalcet for these patients.

In conclusion, the use of cinacalcet to treat severe SHPT is likely to be cost-effective only for those who cannot undergo surgery for medical or personal reasons. Further studies are needed to provide the validity of our simulation model and develop more efficient and cost-effective strategies for treating SHPT in dialysis patients.

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SUPPLEMENTARY MATERIAL

Table S1: Choice of distribution.

Table S2: Cost-effectiveness of cinacalcet without considering the effect on serum calcium and phosphorus levels.

Table S3: Cost-effectiveness of cinacalcet when concurrent intravenous active vitamin D changed to oral administration.

Table S4: Cost-effectiveness of cinacalcet when surgical parathyroidectomy indicated for patients with intact PTH >800 pg/mL.

Figure S1: Probabilistic sensitivity analyses for those eligible for parathyroidectomy, including (A) scatter plot and (B) cost-effectiveness acceptability curve.

Figure S2: Probabilistic sensitivity analyses for those ineligible for parathyroidectomy, including (A) scatter plot and (B) cost-effectiveness acceptability curve.

Item S1: Methods for choice of distribution.

Note: The supplementary material accompanying this article (doi:10.1053/j.ajkd.2011.12.034) is available at www.ajkd.org

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