Study design

This single-center case series study was conducted at the Kaohsiung Veterans General Hospital, a tertiary medical center in southern Taiwan. Ethical approval for this pilot study was provided by the independent ethics committee of the Kaohsiung Veterans General Hospital (VGHKS18-CT-19), and written informed consent was obtained from all patients. All procedures performed in this study involving human participants were in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study is registered with the ISRCTN registry, number ISRCTN92563400.

Subjects

The patients with ESRD who were undergoing dialysis with severe hyperparathyroidism and low bone mass, and who were scheduled to receive denosumab (Prolia; Amgen Inc., Thousand Oaks, CA, USA) were enrolled (denosumab group); The patients with ESRD who had severe secondary hyperparathyroidism owing to ongoing dialysis, and were undergoing annual physical examinations were recruited to form the control group. The denosumab group was screened for eligibility up to 3 months before the study treatment, and baseline evaluations were conducted on day -1 before treatment. In accordance with the regulatory guidelines, patients were enrolled in peritoneal and hemodialysis groups. The key inclusion criteria were age older than 18 years, available laboratory test results at screening and baseline, and clinically acceptable physical examination and electrocardiograph results at screening. The study protocol was amended to include requirements for daily supplementation of calcium and calcitriol based on the standard dialysis guidelines for all subjects with ESRD, low bone mass, and a history of intact parathyroid hormone (iPTH) >800 pg/mL. All patients who had been undergoing renal replacement therapy had various degrees of osteopenia, which was evaluated using dual-energy X-ray absorptiometry (DEXA). Furthermore, all patients in the denosumab

group had forearm, femoral neck, or lumbar spine T scores of <-2.5 of the standard deviation, indicative of low bone mass. The key exclusion criteria were known sensitivity to any study treatment, unstable medical conditions including prior myocardial infarction, history of malignancy, active infection, pregnancy, lactation, nursing, parathyroidectomy with parathyromatosis, drug abuse at screening, and aluminum levels >20 μg/L.

Denosumab group

The denosumab group received a single 60-mg subcutaneous dose of denosumab (Prolia; Amgen, Inc., Thousand Oaks, CA, USA) on day 1. Concomitant medications received included acetaminophen, nonsteroidal anti-inflammatory drugs, vitamins, and treatments for renal disease or its associated conditions. All patients underwent follow-up examinations every month thereafter. During each examination, adverse events were recorded and blood samples were obtained to measure serum calcium, phosphate, and alkaline phosphatase (AP) levels. Patients with severe secondary hyperparathyroidism with iPTH >800 pg/mL and not receiving denosumab were recruited as controls; they underwent BMD and cardiac computed tomography evaluation at 6-month intervals. We only matched the sex and PTH (serum PTH levels in the past three months). After enrolling into the study, the blood pressure, serum PTH and biochemistry were re-evaluated on day zero. In Taiwan, under the bundled payment scheme providers would, on average, receive US $120 dollars per dialysis treatment per patient. This would include the actual cost of dialysis, the cost of providing all injectable medications or their oral equivalents (erythropoietin stimulating agents, iron, phosphate binders, and vitamin D), and the cost of dialysis-related laboratory tests. Hence, only phosphate binders such as calcium carbonate, calcium acetate, and aluminum hydroxide could be prescribed, while cinacalcet and non-calcium phosphate binders had to be self-paid. None of the patients in this cohort received sevelamer, fosrenol, or cinacalcet during the study period.

Measurements

Patients were then treated with calcitriol (1 ug/day), calcium carbonate (3 gm/day) and dialysate of calcium 3.5 mEq/L during the first week after denosumab administration. Our strategy intended to maintain a neutral calcium balance with limited supplementation with active vitamin D, calcium, and appropriate dialysate calcium. We tapered the doses of calcium dialysate from the second week to ensure calcium and later dialysate calcium levels of 3.0 and 2.5 mEq/L, respectively, as far as practicable. The BMD was measured using the DEXA scanner (Hologic Inc., Bedford, MA, USA). The scanned images included four lumbar vertebrae (L1 to L4) and the right proximal femur. The results were presented in g/cm^2 and were derived by dividing the bone mineral content of hydroxyapatite in each region by the projected bone area. To overcome interpatient measurement errors, we used the Hologic scanner, which could automatically adjust for body size, and performed the procedure in a standard body position. The data were analyzed using in-hospital least significant change values (spine: 0.022326 g/cm^2; femoral neck: 0.026675 g/cm^2).

Computed tomography data acquisition

In the absence of known contraindications, beta-blocker medication (metoprolol 50–100 mg; Selokeen; AstraZeneca, London, UK) was administered orally before cardiac computed tomography (CT) in cases where the heart rate exceeded 65 beats per minute. All scans were performed on a 64-detector row CT scanner (Aquilion 64; Toshiba Medical Systems Corporation, Otawara, Japan). The cardiac CT protocol included a nonenhanced electrocardiography (ECG)-gated scan for CAC. The scanning parameters for quantification of CAC were as follows: prospective ECG-triggered sequential mode, tube voltage: 120 kV, tube current: 250–300 mA, gantry rotation time: 0.35 s, detector row width: 0.5 mm, and 180-mm field of view. The images were automatically

reconstructed at 75% of the R–R interval using a 512 × 512 matrix, medium soft-tissue kernel, and 3.0-mm slice thickness with an increment of 3.0 mm.

Data evaluation

Offline CT data analysis was performed by a CT technologist with 15 years of experience performing cardiac CT, and second read by a cardiothoracic radiologist (MTW) with 22 years of experience. Both were blinded to the patient group while performing the analysis. Offline analyses of CT datasets were performed on a validated commercial program (Calcium score; Vital Imaging Medical Group, Anaheim, CA, USA). The segmentation of calcified plaques was based on a semi-automated algorithm known as the regional growing method for efficient assignment of each calcified plaque . The entire coronary arterial tree was inspected for the presence of calcified plaques. The number of calcified plaques and their locations in the four major coronary arteries were recorded. A calcified plaque was defined as an area of three connected pixels with CT attenuation >130 HU on application of three-dimensional (3D) connectivity criteria .

Expected CAC and Rated Progression

The following progression parameters were defined: (absolute [untransformed) CAC change [Absolute], Square root CAC change [Root] and log(CAC+1) change [Log]).

Berry criterion CAC: CACbaseline=0: CAC 6 m>0; CACbaseline=1–100: (CAC6m–CACbaseline) ≥10; CACb >100: (CAC6m–CACbaseline)/CACbaseline ≥10%.

Square root CAC change [Root]: Hokanson criterion: (√CAC6m–√CACb) >2.5;

modified Hokanson criterion: (√CAC6m–√CACb) >2.0.

Modified Raggi criterion: CACbaseline >0: (CAC6m–CACbaseline)/CACbaseline >10% per 6 months.

Log (CAC+1) change [Log]): (log[CAC6m+1]–log[CACbaseline+1]).

Study endpoints

The primary study endpoints were changes in calcium, phosphate, AP concentrations, and changes in CAC from baseline, as evaluated by CT. The progression group comprised patients whose CAC Agatston scores increased according to the Berry criteria or modified Raggi criteria after denosumab therapy; patients whose CAC scores increase was below the Berry criteria or modified Raggi criteria threshold after denosumab therapy formed the non-progression group. The initial data collected from both groups before treatment were compared to identify the predictors for CAC change. The safety was evaluated based on reports of adverse events.

Statistical Analyses

The primary outcomes of interest were changes in CAC, calcium, phosphate, and AP levels. The secondary outcomes included factors affecting changes in CAC scores. All values have been presented as the mean ± standard error of mean. Baseline laboratory and CAC measurements were performed, and the patient characteristics were recorded. The normality of data distribution was checked using the Shapiro-Wilk test. To test the differences between groups, we evaluated ordinal data using the χ^2 or Fisher's exact tests, as appropriate; the t- and Mann-Whitney U tests were employed for analyzing continuous normally distributed and non-normally distributed data, respectively. Differences in the same groups were analyzed using the Wilcoxon signed-rank test. Statistical analyses were performed using statistical software (SPSS version 13; SPSS, Chicago, IL, USA). All tests were performed with a two-tailed significance level of 0.05.