CHAPTER III

METHODOLOGY

Study design

Double-blind randomized controlled trial

Setting

Division of nephrology, Faculty of Medicine, Chulalongkorn University, Bangkok,Thailand.

Target population

Inclusion criteria

1. Chronic renal failure patients with glomerular filtration rate less than 5 ml/min and undergoing CAPD treatment
2. Aged 20-60 years

Exclusion criteria

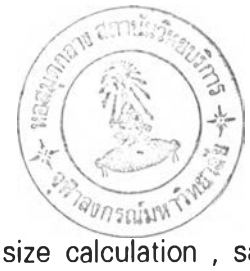
1. Diabetic patients
2. Steroid treatment patients
3. Previous treatment of vitamin D in the past 6 months

4. Post renal transplantation
5. Tuberculosis or other granulomatous disease
6. Hypercalcemia (plasma calcium > 11 mg/dl)
7. Hyperphosphatemia (plasma phosphate > 4.8 mg/dl)
8. Post parathyroidectomy
9. Postmenopausal women.
10. Patients who received H2-antagonist drug which can suppress parathyroid function
11. Aluminum bone disease

Serum aluminum was evaluated before the study. Serum values below 50 ug per liter essentially excluded aluminum toxicity while values above 300 ug per liter confirmed the diagnosis. In cases of serum values between 50 and 300 ug per liter the deferoxamine challenge test was done. The levels more than 350 ug per liter confirmed the presence of biologically significant aluminum accumulation and the patients were exclude from the study (Andress, 1993).

Sample size calculation

The sample size was calculated by the reference of the study of Watson (1989). A prospective trial of 1-alpha-hydroxycholecalciferol therapy was studied in 12 CAPD patients . After treatment with vitamin D ,the levels of PTH were 18 and 73 units in experimental and control groups respectively. In this study the patients were divided into two independent groups and the outcome measurement was mean PTH levels. The different of mean levels of PTH between two independent groups (Mc-Mt) was 55 units (73-18). The standard deviation of first and second



groups were 28.51, 13.39 units respectively. In sample size calculation , sample size (number of cases per group) is ((Za+Zb)V)2/(Mc-Mt)2 (Za = Z value when type 1 error 0.05 , Zb = Z value when type 2 error 0.10 , V = variance of mean levels of PTH , Mc = mean levels of PTH in control group, Mt = mean levels of PTH in treatment or experimental group) . In calculation the sample size from this data we found that,the smallest sample size need was 4 patients per group.

Allocation

All eligible subjects were randomized allocated to experimental and control group by using simple randomization technique.

Method

All 8 patients were performed four daily exchanges, using four 2 liter bags of 1.5-4.25% glucose dialysate during the day and night. The calcium concentration in the bag was 3.5 meq/L. All were on free diet with mean daily intake of 800-1000 mg of calcium and 1.5 gm/kg of protein. Calcium carbonate compound (Chalk Cap R) was prescribed in attempt to maintain serum phosphate level below 5.5 mg/dl and serum calcium over 8.5 mg/dl. If hypercalcemia occurred, aluminium hydroxide or low calcium dialysate (Ca < 2.5 mg/L) were prescribed. All the patients were asked to come to the hospital every month for six months. In every visit, they were assessed for clinical of renal osteodystrophy and soft tissue calcification by complete history taking and physical examination. Blood examinations for parathyroid hormone were taken at the first visit and repeated every 2 months. Blood examination for baseline CBC, BUN, Cr, Electrolytes, LFT, Calcium, Phosphate were taken at the first visit and were repeated every month.

Calcitriol group

The subjects in calcitriol group received one capsule of 0.25 ug of 1,25 (OH)2 D3 (Calcitriol) daily for 2 months. After 2 months, the titrated doses of medication were given depending on serum calcium. Every month, if plasma calcium remained below 9 mg/dl (hypocalcemia), the dosage of calcium carbonate was increased as well as calcitriol. The desired levels of plasma calcium were between 9 to 11 mg/dl. If plasma calcium levels were more than 11 mg/dl the medication was decreased to one capsule every other day, and low calcium dialysate was used. In the next month if plasma calcium levels were still high, the medications were stopped.

Control group

The subjects in control group received capsules of inert substance as placebo for 2 months. The capsules were the same in size, shape, color and weight as calcitriol capsules. After 2 months the tritration method of the medications was done as in the calcitriol group. Each subject had their owns identification number for this study. No other investigator except the one who made the randomized scheme knew the identity of the intervention in each subject.

Outcome measurement

For the primary research question , the outcome measurement was parathyroid hormone levels. According to the secondary research questions, the other secondary outcomes needed to be measured were the levels of calcium,

phosphate and x-ray finding of soft tissue calcificaiton. Progression of hyperparathyroid bone disease was measured by the increasing levels of alkaline phosphatase and typical x-ray features of high turnover bone disease.

Plasma Ca, Al and Mg were measured with an atomic absorption spectrophotometer (Perkin Elmer 1100 B Atomic absorption Spectrophotometer : normal values 9 - 11 mg/dl , 0 - 50 ug/L and 1.7 - 2.8 mg/dl respectively). Plasma phosphate and alkaline phosphatase were measured by colorimetric methods (normal values 2.5 - 4.8 mg/dl and 98-279 u/l ,respectively). Plasma Na and K were measured by flame photometer (Corning 480 ; Flame photometer , normal values 135 - 150 meq/l and 3.5-5.0 meq/l respectively) . Plasma Cl was measured by chloride fluorometric (Corning 480 ; Flame Photometer : normal value 95 -105 meq/l). Plasma HCO3 was measured by HCO3 gas detector (Carbondioxide analyzer : normal value 20 - 30 meq/l) . Plasma BUN was measured by enzymatic method of Human (normal value 5 - 20 mg/dl) .Plasma Cr was measured by Jaffee reaction (normal value 0.5 -1.2 mg/dl) . Parathyroid hormone was measured by immunoradiometric (IRMA) assay of intact hormone (Incstar corporation USA kit XR 080082 , normal value 13-54 pg/ml). Serum ferritin concentrations were measured by immunoradiometric assay Ferritin IRMA (RIA: Gamma 1282 Compugamma : normal value 10 - 200 ng/ml). Serum iron was measured by spectrophotometer (normal value 100 - 170 ug/dl).

A complete series of skeletal radiography (hand-AP, shoulder-lateral, skull-lateral, spine-lateral, pelvis-AP, chest, abdomen, distal arm and leg) were taken at the first visit. The follow-up pictures were taken after 3 months to search for the evidences of renal osteodystrophy or soft tissue calcification.

The termination of the study

The codes planed to be broken only at the time of data analysis. In an emergency situation such as uncontrolled hypercalcemia (Ca > 11 mg/dl), hyperphosphatemia (P > 4.8 mg/dl), evidence of soft tissue calcification or drug allergy, the medications were stopped and the results were recorded.

Data summary and analysis

Descriptive data was summarized in mean and SD. The differences between experimental and control group were tested by Student unpaired t-test. The differences between pre-treatment and post-treatment were tested by Student paired t-test. The statistical significant level (P-value) was set below 0.05 (two-tailed).

Summary

In this chapter , and overview of the research methodology as well as the criteria for termination of the study has been provided. Also, the plan for data analysis has been described.