CHAPTER III

METHODS

This study was carried out (1) to determine the efficacy and safety of oxymetholone 50 mg twice daily in end-stage renal disease undergoing maintenance hemodialysis in terms of lean body mass, (2) to assess the relationship between change in lean body mass and insulin resistance, and (3) to determine the adverse events in short term use.

This chapter describes in detail how the study was performed. It is divided into three sections; the first section describes the study site and the patients in this study including patient selection, sample size estimation, and patient randomization. The second section explains the methods including study design and procedures, laboratory measurement. The third section details statistical analysis.

1. Study Site and Patient

1.1 Study Site

This study was conducted at the hemodialysis unit, The Kidney Foundation of Thailand at Kalayaniwattana building, Priest hospital, and all laboratory measurements were monitored at Phramongkutklao hospital.

1.2 Patient Selection

Study subjects were the end-stage renal disease patients undergoing maintenance hemodialysis in the hemodialysis unit of The Kidney Foundation of Thailand at Kalayaniwattana building, Priest hospital between June 2006 to February 2007. The patients who met the following criteria were recruited in the study.

Inclusion Criteria

1. Patients 20 years old or more
2. Undergoing hemodialysis at least 3 months
3. Regularly undergoing hemodialysis, which referred to missing hemodialysis session no more than 2 sessions per one month in the past 6 months
4. Adequate hemodialysis, which referred to Kt/V > 1.2 per week
5. No treatment with androgen or steroid within 6 months before starting the study
6. Normal liver function, which referred to AST less than 105 U/L and ALT less than 120 U/L
7. Non-diabetic, which referred to fasting blood glucose less than or equal to 126 mg/dL and not taking any diabetic medications or receiving any dosage forms of insulin
8. Written informed consent

Exclusion Criteria

1. Patients known to be malignancy
2. Patients with chronic infection e.g., tuberculosis active within 1 year before the study

3. Patients with acute infection or illness within 2 weeks before the study
4. Patients with immunological disorders e.g., lupus, HIV or on immunosuppressant medications such as steroids
5. Patients undergoing operation or surgery within the last six months
6. Patients with hematocrit less than 28%
7. Patients who are pregnant or lactation

If the patients had an intolerable adverse event, serum AST, ALT elevated more than 3 times the upper limit of normal, and hypersensitivity to oxymetholone during the study period, these patients would be excluded.

1.3 Sample Size Estimation

The sample size was determined by using change in lean body mass as the primary outcome measure and by extrapolating expected changes and standard deviation from data in the study of the effect of oxymetholone in continuous ambulatory peritoneal dialysis (CAPD) patients *(26)*. An increase in lean body mass of 88.9 gram/kilogram (g/kg) was considered to be a significant improvement and the mean standard deviation of 68.6 g/kg. The target sample size was 13 subjects per group that will provide 80% power to detect a significant effect, using a 2-sided, p=0.05. With an anticipated dropout rate of 10%, the sample size was set at 15 subjects per group.

Formula :

$$n = 2\left[\frac{(Z_\alpha + Z_\beta)\sigma}{\Delta}\right]^2$$

Determination

n = number of study subject in each group
α = 0.05 (two-sided) ; $Z\alpha = 1.96$
β = 0.1 (one-sided) ; $Z\beta = 1.28$
σ = mean standard deviation (SD) from data in patients with continuous ambulatory peritoneal dialysis (S.D.)
= 68.6 g/kg
Δ = changes in lean body weight (gram/kg) between two groups is 88.9

Replacing all the parameters

$$n = 2\left[\frac{(1.96+1.28)68.6}{88.9}\right]^2$$

= 13 subjects

Expect 10% drop out $\quad n = \dfrac{13}{(1-0.1)} \approx 15$ subjects

Therefore, 30 patients were recruited for this study (15 subjects per group).

1.4 Patient Randomization

Forty-three patients were randomly assigned equally into two groups in a 1:1 manner using block of four randomization. Oxymetholone and a placebo that was identical in appearance to the active drug were prepared by a British dispensary company. One group received oxymetholone 50 mg twice daily, in the morning and in the evening, for 24 weeks and other group received placebo twice daily at the same time for 24 weeks. Then, simple random sampling was used to allocate the sequence of patient who would be assigned to the control and the study group. Thus, randomzied 11 blocks were applied.

2. Methods

2.1 Study design and Procedures

All patients gave written informed consent for study participation and the protocol was approved by the Ethic Committee on Human Research at Phramongkutklao hospital on February 20th, 2006.

2.1.1 Materials

The following materials were used to examine and record patients' data and included the study and placebo sample.

- Dual-energy X-ray absorptiometry (DEXA) scan (Hologic QDR-4500W)
- Hemodialysis patient profile (Appendix A)
- Medication sheet (Appendix B)
- Biochemical laboratory form (Appendix C)
- Record of body weight (Appendix D)
- Record of hematocrit and erythropoietin administration (Appendix E)
- Adverse drug event form (Appendix F)
- Naranjo's algorithm (Appendix G)
- 36-item short form of the Quality of Life Score (SF-36) (Thailand version 1.0) (Appendix H)
- Dietary record (Appendix I)
- Consent form (Appendix J)
- Research subject information sheet (Appendix K)
- Oxymetholone 50 mg
- Placebo, which was mainly compose of lactose

2.1.2 Procedure

This study was a block randomized, placebo-controlled trial study design. All subjects were recruited from the hemodialysis unit, The Kidney Foundation of Thailand at Kalayaniwattana building, Priest hospital. The following steps were taken.

(1) Select a patient who met the entry criteria. Subject eligibility was determined by laboratory data, which included hematocrit, AST, and ALT, patient interview, and chart review. All eligible patients were invited to participate in this study. After both verbal and non-verbal description of the study, patient provided written consent form for study participation. The patients have been given a detailed explanation of the study and asked for blood measurement.

(2) The patient demographic data and laboratory data were collected from the patient folders needed for analysis and 3-month retrospective laboratory data that reflect nutritional status, past illness. After that patients were visited by the researcher as following.

First visit: Patients were interviewed about past medical history, present illness and medication history on the day after hemodialysis in the middle of the week. The demographic data and the current medication were recorded in the hemodialysis patient profile (Appendix A) and medication sheet (Appendix B), respectively. Dry weight, hematocrit level and erythropoietin administration were obtained from patient folders, which were found at hemodialysis unit. All these three data were recorded in record of body weight and record of hematocrit and erythropoietin administration (Appendix D and Appendix E, respectively).

On the first visit, blood was collected after 12-hour overnight fast for base line measurements and drawn for 30 milliliters. Blood was analyzed for hematological parameter and biochemical parameter before receiving either oxymetholone or placebo. All parameters were recorded in biochemical laboratory form, which is included as following (Appendix C).

- Complete blood count (CBC)
- Fasting blood glucose
- Fasting serum insulin
- Blood urea nitrogen and serum creatinine
- Serum albumin
- Lipid profile (including Cholesterol, HDL-C, LDL-C, Triglyceride)
- Dual Energy X-ray Absorptiometry (DEXA scan)
- Liver function test (including AST, ALT, total bilirubin, direct bilirubin)
- Hormonal test (e.g., PSA, LH, and testosterone)

Second visit: On the day after hemodialysis in the middle of the week

- Weight was determined on an electronic scale without their shoes. These values were called dry weight. After that, the values were recorded in record of body weight (Appendix D)
- Height was measured using a standard stadiometer. Patients stood, without their shoes, erect on the floor board of the stadiometer with his or her back to the vertical backboard of the stadiometer.
- Body composition was assessed immediately after dialysis. Dual energy x-ray absorptiometry (DEXA) scan was used to measure lean body mass and fat mass in grams using a whole-body scan.
- Self-report functioning was measured using the score of the SF-36 (Appendix H).
- Chest x-ray and electrocardiogram (ECG) were performed to rule out cardiac problems
- Ultrasound scan was performed to determine internal organ dysfunction.

Third visit (week 0): Patients were assigned into two groups after hemodialysis. Individual treatment group assignment was based on a block randomization method using a 1:1 manner random block number of computer-generated sequences. Individuals were randomized into one of two groups.

- Oxymetholone group: subjects were assigned to receive oxymetholone 50 mg per oral in the morning and evening for 6 months. Patients were appointed to check out their hematological and biochemical parameters including liver function test monthly until the study finished.
- Control group: subjects were assigned to receive placebo 1 tablet per oral in the morning and evening for 6 months. Then, patients were followed-up monthly as study group.

(3) Side effects such as acne, alopecia, hirsutism and so forth are evaluated biweekly by asking after individuals received their treatment of study medication (Appendix F). Also, adverse drug reaction (ADR) probability scale was assessed by using Naranjo's algorithm (Appendix G).

(4) Erythropoietin administration was adjusted depending on hematocrit as following:
- If hematocrit is $\leq$ 33%, erythropoietin will be administered twice a week;
- If hematocrit is 34-36%, erythropoietin will be administered once a week;
- If hematocrit is $\geq$ 36%, erythropoietin will be omitted.

(5) Patients were advised to walk at least 1 kilometer a day. This activity was determined by self-report.

(6) Caloric intake and energy were evaluated from three-day dietary records by the dietitian. Patients were educated how to fill in the records by the dietitian.

(7) After taking either oxymetholone or placebo for six months, the patients were physically examined as done in the first and second visit (number 2). The same equipment was used for evaluations for all patients. The diagram of the study procedure is shown in Figure 6.

Treatment was halted when liver toxicity occurs (AST and/or ALT more than three times the limit of normal) and resumed when the adverse effect disappears.

2.1.3 Laboratory Measurement

The summary of all measurement is shown in Table 14. Patients were followed up at week 4, 8, 12, 16, 20, 24 by

- A liver function test, which included ALT, AST, total bilirubin,and direct bilirubin
- Nutritional status, which included serum albumin level
- Hematological parameters, which were hematocrit and hemoglobin

At week 8, 16, 24, lipid profiles were examined, which were cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride (Appendix C). The blood samples were taken from blood line before a dialysis session in the middle of the week. Patients were notified in advance before the blood was taken.

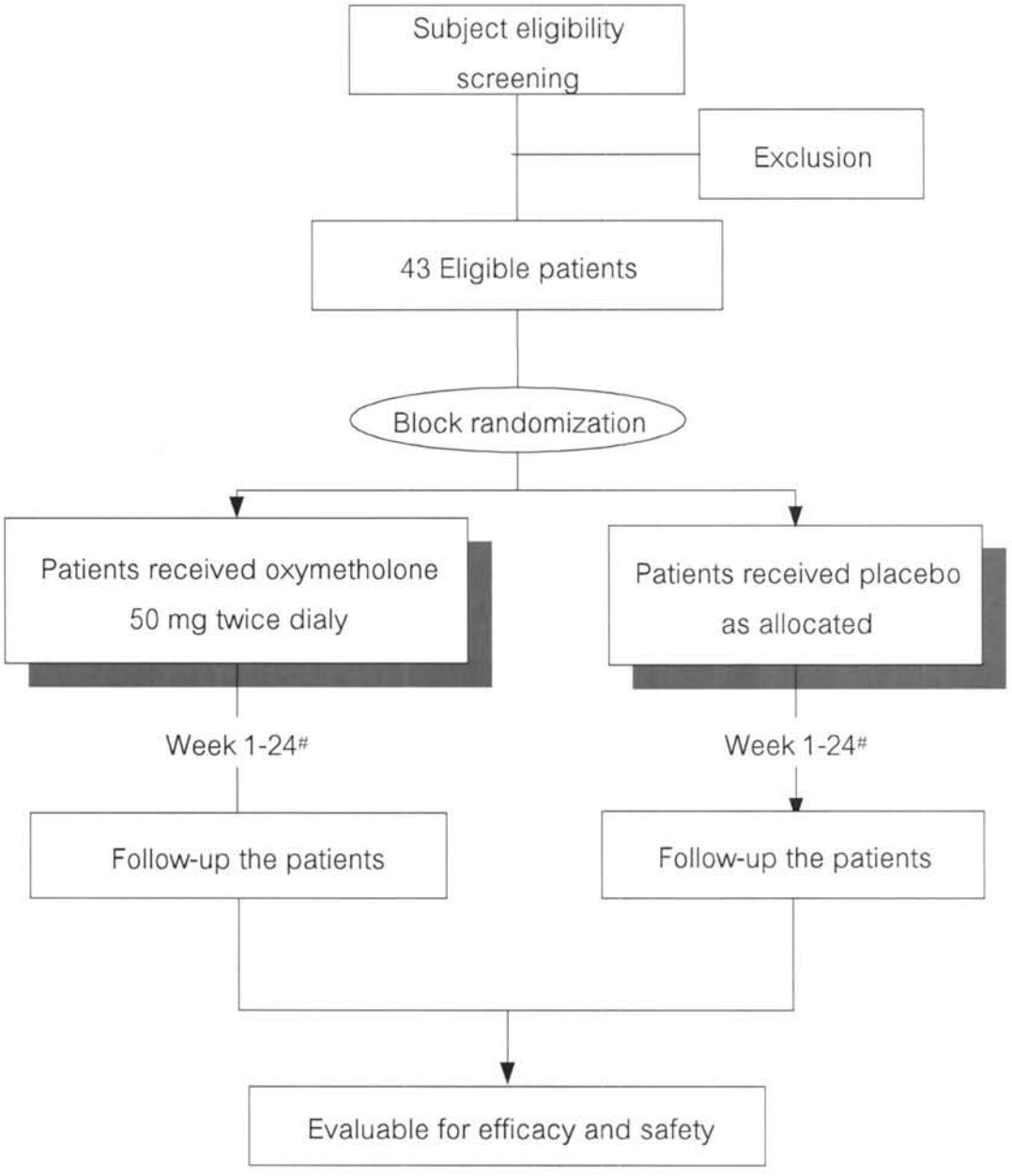
To evaluate insulin resistance, the Homeostatic Model Assessment of insulin resistance (HOMA-IR) was described by Matthews et al. in which *(103)*

$$\text{HOMA- IR} = \frac{\text{fasting serum insulin } (\mu\text{U/mL}) \text{x fasting plasma glucose (mg/dL)}}{405}$$

Table 14 Summary of all monthly patient examinations

Parameter	Pre study	Duration					
		Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Pre study							
1.ECG	✓						✓
2.U/S scan	✓						✓
Efficacy							
1. Dry weight	✓	✓	✓	✓	✓	✓	✓
2.Anthropometry	✓	✓	✓	✓	✓	✓	✓
3. URR	✓	✓	✓	✓	✓	✓	✓
4. Food record	✓	✓	✓	✓	✓	✓	✓
5. Hct and Hb	✓	✓	✓	✓	✓	✓	✓
6. BUN	✓	✓	✓	✓	✓	✓	✓
7. Albumin	✓	✓	✓	✓	✓	✓	✓
8. DEXA scan	✓						✓
9. FBS	✓						✓
10. Insulin resistance	✓						✓
Adverse event							
1.ALT	✓	✓	✓	✓	✓	✓	✓
2.AST	✓	✓	✓	✓	✓	✓	✓
3.AP	✓	✓	✓	✓	✓	✓	✓
4.GGT	✓						✓
5.T. bililubin	✓						✓
6.D. bililubin	✓						✓
7.Lipid profiles	✓		✓		✓		✓
8.Clinical signs and symptoms		✓	✓	✓	✓	✓	
Concurrent medication	✓	✓	✓	✓	✓	✓	✓

U/S = ultrasound, BUN = blood urea nitrogen, URR = urea reduction ratio, FBS = fasting blood sugar



Note: # means week 24 after taking on any treatment

Figure 6 Diagram of the study procedure

2.2 Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software version 11.5. Intention-to-treat analysis was used by replacing the missing data with series mean for each group. Both descriptive and inferential statistics were determined. A bi-directional α-level of significance was set at $p= 0.05$ for all measures.

- Descriptive statistics was used for demographic results which are expressed as mean (± standard deviation; SD) and percentage unless otherwise indicated.
- Chi-square tests were used for evaluating categorical variables.
- Continuous variables between study and control groups were compared with unpaired *t*-tests.
- Continuous variables between baseline and at the end of study for each group of patients were compared by using paired student *t*- tests.
- Analysis of covariance (ANCOVA) was used for controlling covariate factors if baseline data are different between the patient groups.
- One-way repeated analysis of variance (ANOVA) was used to examine the main effects of time when comparing data obtained at one time to data obtained at another time during the study period.
- One-way repeated analysis of variance (ANOVA) with between-subjects factor was used to determine the main effects of overall difference between the control and the study groups with respect to the mean of the data.
- Univariate correlations were evaluated using Pearson correlation analysis.