CHAPTER 3

MATERIALS AND METHODS

Materials

1. Instruments and materials used for detecting blood levels of theophylline
   - Discrete clinical analyzer aca® IV (Dupont USA)
   - Centrifuge (Quantum)
   - Refrigerator
   - Digital micro pipette (100-1,000 ul)
   - Pipette 1 ml, 5ml
   - Test tube
   - Test tube rack
   - Sample cup
   - Cup lid
   - Diluent reagents
     - Purified water diluent (3.5 L)
     - Amp. HCI Buffer (1.7 L)
     - Phosphate Buffer (1.7 L)
     - Tris. HCI Buffer (1.7 L)
     - Glycerine Buffer (1.7 L)
     - Cell wash solution (500 ml)
   - Calibrater reagents
     - Theophylline calibrator
   - Control reagents
     - Control for theophylline

       QCS® 1 (Ciba – Corning)

       QCS® 2 (Ciba – Corning)

- Absorbance test solution ABS
- Analytical test packs
  - ABS Absorbance Test
  - Theophylline test packs
- Thermal printer paper
- Latex examination gloves

2. IPD chart
3. Kardex card
4. Theophylline dosage program
5. Theophylline Drug Monitoring record form
6. Therapeutic Drug Monitoring Report

Methods

1. Study design

Prospective randomized parallel design was employed in the study. Subject entries were randomly assigned into either the study or the control group which include the equal number of subjects. The details are described in the following sections.

2. Subjects

Patient selection: Thai patients with respiratory disorders admitted to Department of Medicine at Maharaj Nakhonsrithammaraj Hospital. Patients included in this study were not critically ill or with diseases affecting theophylline Cl such as acute cardiogenic pulmonary edema, viral illness, hepatic cirrhosis. All patients were treated with theophylline under the trade name of Theodur® alone or together with other drugs

(beta-adrenergic agonists, corticosteroids and anticholinergic drugs), and did not receive the drugs affecting theophylline Cl except drug in the study. The age of patients was equal to or greater than 18 ys.

Sample size for the study was calculated as follows:

Beginning with t statistic (Hamburg M. and Young P., 1994):

$$t = \frac{P1 - P2}{SE}$$

where, $P1$ is the incidence rate of patient who have theophylline level in toxicity range while receiving usual dose of theophylline by the medical doctors in Maharaj Nakhonsrithammaraj Hospital : retrospective study P1 = 0.3, $P2$ is the incidence rate of patient who have theophylline level in toxic range while using the program for calculation for dose of theophylline : Literature report $P2$ = 0.2, t is a student-t statistic analytical at 95% confidence by one tailed test (t = 1.64), and SE stands for standard error:

$$SE = \sqrt{P1P2(\frac{1}{n1} + \frac{1}{n2})}$$

where, $n1$ = sample size in the control group

$n2$ = sample size in the study group

The number of samples of the control group is set to equal to those of the study group given Null and alternative hypothesis as follows:

H0 , $P2 \geq P1$

H1 , $P2 < P1$

One is able to obtained the sample size by the following calculation

$$1.64 = \frac{(0.3 - 0.2)}{\sqrt{\frac{2 * 0.2 * 0.3}{n}}}$$

$$n = 32.28 \approx 32$$

Sample size: At least 32 patients in each group

3. Procedure of theophylline monitoring in medical ward

3.1 Dosage regimen and administration

Dosage regimen of theophylline is different in the control group and the study group

3.1.1 The control group

36 patients recieved theophylline which were prescribed by physicians in department of Medicne in Maharaj Nakhonsrithammaraj Hospital.

3.1.2 The study group

36 patients recieved theophylline calculated by pharmacist according toTheophylline dosage program (Appendix D) and prescribed by physicians in department of Medicine in Maharaj Nakhonsrithammaraj Hospital.

3.2 Blood sample collection

Theophylline serum concentration was considered to achieve steady state after the fixed dosage regimens of the drug were given to the patients for at least two days. Blood sample for determination of serum theophylline concentration was drawn (3 ml) and sampling time was 6 hour post dose (Research and Development Laboratories, 1980).

3.3 All of available patients' data related to the study were recorded; including age, gender, weight, height, medical history, smoking history, diagnosis, drugs administered, dosage regimen, duration of theophylline therapy, factors affecting theophylline elimination, clinical response, ADRs and laboratory data.

Theophylline serum level was monitored once in each patient who received Theodur® for about 2-3 days (depend on theophylline elimination in each patient). Relevant clinical signs, symptoms or theophylline adverse reactions were monitored. These patients were followed-up until discharged. If theophylline level was in subtherapeutic or toxic range, the corresponding physician who took care of the patients will be informed immediately.

4. Therapeutic monitoring of theophylline

All patients treated with thophylline were monitored for theophylline serum levels, clinical response and ADRs.

4.1 Clinical response of theophylline includes decreasing of wheezing, dyspnea, cough, rale, sputum and other symptoms.

4.2 Adverse Drug Reactions were listed as follows:

- Central nervous system: headache, dizziness, nervousness, insomnia, seizure.
- Gastrointestinal tract: nausea, vomiting, abdominal pain, and diarrhea.
- Cardiovascular system : pulse rate > 100 bpm, muscle tremor, ventricular arrhythmia, hypotension, sinus tachycardia.

Examples of record form for patient's data and theophylline serum concentration measurement were demonstrated in Appendix A, B, C, D, E, F, and G

5. Analytical method

Theophylline level in serum samples was determined by the so-called THEO method. Its principle was immunoassay method using fluorescence polarization technique (aca® IV Dupont USA). The THEO method is based on a particle-enhanced turbidimetric inhibition immunoassay (PETINA) adapted to the aca® analyzer. The THEO method uses a single-pack rate technique to measure theophylline. The THEO pack contains a particle reagent (PR) which is a latex particle with theophylline linke

to the surface. Aggregates of these particles are formed when a theophylline specific monoclonal antibody (Ab) is introduced. Theophylline present in a sample completes with the particles for the antibody, thereby decreasing the rate of aggregation. Hence the rate of aggregation is inversely propotional to the concentration of theophylline in the sample. The rate of aggregation is measured turbidimetrically at 340 nm. The concentration is determined by means of a previously prepared lot-specific calibration curve or mathematical function:

Theophylline + PR+ Ab ⟶ PR-Ab complex + Theophylline -Ab

(Absorbed at 340 nm)

It has been claimed that a split sample comparison between the method and HPLC procedure showed a good correlation. Prior to assay, calibration was routinely performed (the detail of aca IV® Dupront USA is in Appendix H)

6. Data analysis

6.1 Chi-square test was employed to compare demographic data and medical conditions of patients between the control group and the study group.

6.2 Comparison between the measured and the predicted theophylline serum concentrations

Paired t-test was utilized to compare the following outcomes:

- The difference between theophylline average level from theophylline dosage program ($C_{predict}$) and measured theophylline level (C_{real}).

Unpaired t-test was utilized to compare the clinical outcome

- The difference of average theophylline level ($C_{predict}$-C_{real}) and measured theophylline level between the control group and the study group.

- The difference of theophylline level between the patients with and without co-disease

6.3 Therapeutic monitoring of theophylline

Chi-square test was utilized to compare the following outcomes:

- Percentage of patients subcategorized as subtherapeutic, therapeutic,and toxic ranges between the control group and the study group.

- The incidence of theophylline adverse reactions between the control group and the study group

- The incidence of theophylline adverse reactions in theophylline level sub-categorized as subtherapeutic, therapeutic, and toxic range between the control group and the study group

- The clinical response between the control group and the study group.

One-way ANOVA was utilized to compare the following outcomes:

- The difference of theophylline level among patients who received theophylline with enzyme inducer / inhibitor, and without enzyme inducer / inhibitor.

6.4 Attempts on correlation testing were done on 2 couple outcome parameters including: theophylline adverse reactions vs. theophylline plasma drug concentrations, and clinical response vs. theophylline plasma drug concentrations