Chapter III

Materials and Methods

Materials

1. **TDX®Digoxin II**

1.1 No. 9511-01, Digoxin Calibrators

Six vials with accurately measured amounts of digoxin in human serum at the following concentrations.

Vial	Digoxin Concentration (ng/ml)
A	0.0
B	0.5
C	1.0
D	2.0
E	3.0
F	5.0

1.2 No. 9511-10, Digoxin Controls

Three vials of digoxin in human serum should read within the following ranges :

Vial	Digoxin Concentration (ng/ml)
L	0.55-0.95
M	1.30-1.70
H	3.15-3.85

(Preservative : 0.1% Sodium Azide)

1.3 No. 9511-01, Digoxin II Precipitation Reagent

(3% 5-sulfosalicylic acid in 50% agueous methanol)

1.4 No. 9511-01, Dilution Buffer

The dilution buffer, a 0.1 M phosphate buffer, contains a protein stabilizer and 0.1% Sodium Azide as a preservative.

1.5 No. 9511-01, Digoxin II Reagent Pack

There are three vials of reagents

Vial	Components
P	Pretreatment solution : Surfactant in buffer (Preservative : 0.1% Sodium Azide)
S	< 1% Digoxin Antiserum (Rabbit) in buffer with protein stabilizer. (Preservative : 0.1% Sodium Azide)
T	< 0.01% Digoxin fluorescein tracer in buffer with protein stabilizer (Preservative : 0.1% Sodium Azide)

2. Apparatus

2.1 Automated Fluorescence Polarization Analyzer (Diagnostic Division, Abbott Laboratories, Inc., Irving, Tx, U.S.A.)

2.2 Centifuge (Model CS, Internal (ICE) Centrifuge International Equipment, Inc. Needham His, Mass, U.S.A.)

2.3 Vortex-Genie (Model K-550 GE, Scientific Industries, Inc., Bohemia, N.Y., U.S.A.)

2.4 Freezer (forma Bio-Freezer, Forma Scientific, Inc., U.S.A.)

Methods

1. Subjects

The subjects studied were Thai patients with heart failure of Medical Department of Chulalongkorn Hospital and were both admitted patients and out patients. Patients included in this study were not in critically ill. Patients were pleased to involve in the study. All patients were treated with digoxin alone or digoxin together with other drugs that used for controlling their present diseases status by traditional physician prescribing practices.

All of available patient data related to the study were recorded; including age, sex, weight, height, medical history, diagnosis, drug administered, dosage regimens, duration of therapy, serum creatinine and other clinical and laboratory data.

2. Dosage Regimen and Administration

Patients were treated with digoxin alone or digoxin together with other drugs such as diuretics, β-blockers , or vasodilators. The dosage regimen was recommended by physician prescribing in general practice of Medical Deparment of Chulalongkorn Hospital and was recorded for individual patients. Dosage regimen of digoxin is commonly 0.125-0.25 mg once a day by oral administration.

3. Sample Collection

The digoxin serum concentration was considered to achieve steady state after the fixed dosage regimens of the drug were given to the patients for at least 5 days. Venous blood sampling was drawn (5 ml) from forearm of the

patient at the appropriate time to determine of serum digoxin concentration. Here, the sample was collected between 8-12 hours after the last digoxin dose.

All the blood samples were allowed to clot and centrifuged immediated (2,000 rpm; 10 minutes; at room temperature). Serum was separated and assayed in the same day that the samples were collected or was frozen until assayed in the next day.

4. Analytical Method

Digoxin concentration was measured by immunoassay using Fluorescence Polarization Technique (TDX® Analyzer System)

4.1 Preparation for Testing Analysis

A pretreatment step must be performed for each digoxin sample (calibrators, controls and patient samples) before testing by the same procedure.

4.1.1 Number a centrifuge tube for each sample and place in a suitable rack.

4.1.2 Set the precision dispenser to dispense 230 mcL. and fill it with Digoxin II precipitation reagent. Dispense 230 mcL of precipitation reagent into each centrifuge tube.

4.1.3 Accurately pipet 230 mcL of the serum sample to be assayed into its corresponding centrifuge tube containing precipitation reagent.

4.1.4 After pipetting all the sampies, cap each centrifuge tube and mix each on a vortex mixer for 3-5 seconds to ensure thorough mixing.

4.1.5 Place the tube into the centrifuge head.

4.1.6 Centrifuge the samples for 5 minutes or until a clear supernatant and a hard, compact pellet of denatured protein is obtained.

4.1.7 After centrifugation is completed, verify presence of protein pellet, uncap each tube and decant the supernatant into the corresponding sample well of a sample cartridge.

4.2 Performing an Assay Calibration

The required items were calibration carousel, cuvettes, sample cartridge, reagent pack, and calibrators.

4.2.1 Preparation of the carousel

- Load 6 cuvettes in positions #1 to #6 in the carousel.
- Load 6 sample cartridges in positions #1 to #6 in the carousel
- Transfer the supernatant from centrifuge tube into sample wells as follows :

Calibrators	Sample wells
A	1
B	2
C	3
D	4
E	5
F	6

4.2.2 Load the carousel in the instrument

4.2.3 Load the reagent pack in the instrument

4.2.4 Close the door of the TDX® analyzer

4.2.5 Press run

4.2.6 The instrument commences operation

4.2.7 Wait for run to complete and keep the printout

4.3 Performing and Assay Controls

The required item were assayed carousel, cuvettes, sample cartridge, reagent pack and controls

4.3.1 Preparation of the carousel

- Load 3 cuvettes in positions #1 to #3 in the carousel
- Load 3 sample cartridges in positions #1 to #3 in the carousel
- Transfer the supernatant from centrifuge tube into sample wells as follows :

Calibrators	Sample wells
L	1
M	2
H	3

4.3.2 Load the carousel in the instrument

4.3.3 Load the reagent pack in the instrument

4.3.4 Close the door of the TDX® analyzer

4.3.5 Press run

4.3.6 The instrument commences operation

4.3.7 Wait for run to complete and keep the printout

4.4 Performing and Assay Samples

The required item were assayed carousel, cuvettes, sample cartridge, reagent pack and controls

4.4.1 Preparation of the carousel

- Load 7 cuvettes in positions #1 to #7 in the carousel
- Load 7 sample cartridges in positions #1 to #7 in the carousel
- Transfer the supernatant from centrifuge tube into sample wells as follows :

Sample number	Sample wells
1	1
2	2
3	3
4	4
5	5
6	6
7	7

4.4.2 Load the carousel in the instrument

4.4.3 Load the reagent pack in the instrument

4.4.4 Close the door of the TDX® analyzer

4.4.5 Press run

4.4.6 The instrument commences operation

4.4.7 Wait for run to complete and keep the printout

5. Monitoring Serum Digoxin Levels

5.1 Serum Digoxin Concentration Evaluation

5.2 Adjusting Digoxin Dosage Regimen by Pharmacokinetic Method.

If digoxin concentrations were not within therapeutic range, the new drug dosage regimen was calculated by the pharmacokinetic equations (Equation 10-11 in Appendix A) and followed up until the desired therapeutic ranges were achieved. The clinical status of the patient was calculated and determined through observation of signs or symptoms which indicated that the disease could not be controlled or digoxin toxicity had occured. Digoxin toxicity was determined by the presence of cardiac arrhythmias, nausea,anorexia,vomitting or headaches.

After the new dosage regimen was calculated, the physician was then informed and the dosing interval was rounded up and adjusted to a figure convenient for dosage administrstion. (Equation 10 in Appendix)

5.3 New Dosage Administration

The patients whose digoxin levels were not within therapeutic range, the dosage regimen was then adjusted.

5.3.1 After the patients were on a new fixed dosage regimen (as described in 5.2) of digoxin for at least 5 days, blood sample was drawn to determine the digoxin concentration by collecting between 8-12 hours after the last dose.

5.3.2 After the digoxin concentration was assayed by Flourescence Polarization Technique (TDX® Analyzer System). If digoxin concentration was not within therapeutic range, the procedures in 5.2 to 5.3 were again performed and followed up until the desired therapeutic range was achieved.

6. Data Analysis

6.1 General evaluation

General characteristics of the patients included in this study were evaluated according to their age, sex, weight and height.

Percentage of patients taking various dosage regimens, percentage of patients taking digoxin alone or digoxin along with various drugs, were determined.

6.2 Evaluation of the patients measured digoxin concentrations

Serum digoxin concentration was evaluated whether it was therapeutic, subtherapeutic or overtherapeutic range. Percentage of patients whose serum digoxin concentration was within each range were determined.

6.3 Observation for any relationship between the serum digoxin levels and the results of the treatments and the side effects or toxicities. Percentage of patients showing clinical improvement and side effect while their digoxin serum concentrations were within therapeutic range, subtherapeutic range or overtherapeutic range, were observed.

6.4 Serum digoxin levels of patients taking digoxin alone and digoxin along with other drugs were observed and compared using ANOVA to determine if any drug interaction might have occured.

6.5 Effect of age on digoxin concentrations was observed through the comparison of digoxin concentrations between the adult and the aged groups. Digoxin concentrations of patients in the adult (20-60 years) and the aged (above 60 years) groups were compared using ANOVA for patients taking different dosage regimens of digoxin, i.e.,0.125 mg. per day, 0.25 mg. per day and 0.125 alternate with 0.25 mg. per day.

6.6 Comparison between the predicted and measured blood levels

The predicted digoxin concentrations were calculated from the available patient data such as age, sex, body weight, height, serum creatinine; using Equation 1-9 (Appendix A).

6.7 The predicted values were compared with the measured values using student t test.

The new measured values of digoxin concentration were calculated using equation 9, in Appendix A, the pharmacokinetic parameters were obtained from their previous measured digoxin concentration.

Correlation between creatinine clearance and digoxin clearance was determined.