# CHAPTER III

# MATERIALS AND METHODS

## Materials

1. Drug

Generic Enalapril (enaril ®) 5, 20 mg from pharmacy department Pramongkultklad Hospital in October 1999 to July 2000 (as enalapril maleate)

2. Instruments

- Mercury Sphygmomanometer
- 24-hour Ambulatory blood pressure monitoring machine (TM-2421,A&D Company limited, Japan)

## Patients

Subjects were recruited into the study based on the follow criteria:

Inclusion criteria

- The patients were men or women with an aged older than 18 years old
- Primary hypertensive with the office seated SBP in the range 140 – 179 mmHg or DBP in the range of 90-109 mmHg at the end of an initial 2-week placebo run-in period (at baseline) or antihypertensive drugs withdrawal and placebo run-in for at least 5 times of half life

- Mean 24-hour ambulatory blood pressure (ABP) showed DBP ≥ 85 mmHg after 2 weeks placebo run-in & washout period able to recruited.
- The patients were willing to be recruited in this study and sign the consent form

Exclusion criteria

- hypersensitivity to enalapril or other ACE inhibitors
- secondary hypertension of any etiologies
- having chronic cardiovascular diseases (e.g. congestive heart failure, myocardial infarction, angina pectoris)
- significantly impaired liver function (AST,ALT ≥ 2 time of normal value)
- significantly impaired renal function (SCr ≥ 265.5 μmol/l or ≥ 3 mg/dl)
- Pregnancy or lactation
- Office seated systolic blood pressure ≥ 180 mmHg or DBP ≥ 110 mmHg during the study period
- Serum potassium < 5.5 mmol/l
- Uncontrolled DM

## Method

*Study design*

After a 2-week placebo run-in and washout from any previous antihypertensive therapy period, office seated blood pressure (OBP) were measured by mercury sphygmomanometer and 24 hour blood pressures were monitored by using ambulatory blood pressure monitoring (ABPM) machine. The patients were eligible for this study if their mean office diastolic blood pressure (DBP) was 90-109 mmHg and their mean 24-hour DBP by ABPM machine was ≥ 85 mmHg. The patients were classified into mild or moderate hypertensive groups according to the levels of their office sitting sytolic and diastolic blood pressures.

- mild hypertensive patients (SBP 140-159 mmHg or DBP 90-99 mmHg)
- moderate hypertensive patients (SBP 160-179 mmHg or DBP 100-109 mmHg)

Mild hypertensive patients were prescribed to start with the dosage of 5 mg of enalapril once daily (OD) morning after meal for 4 weeks, and their OBP and 24-hour ABPM were evaluated ,if their office DBP remained ≥ 90 mmHg after finishing this 4 weeks period the patients would be prescribed to receive the higher dosage of 10 mg once daily morning after meal (10 mg enalapril obtained from 2 tablets of 5 mg/tab or half tablet of 20 mg/tab). In contrary, patients whose office DBP were decreased to < 90 mmHg, the dosage of enalapril would be adjusted to 2.5 mg OD. Their blood pressure were again evaluated after the patients were on the new dosage regimen for at least 4 weeks

Moderate hypertensive patients were treated with 10 mg enalapril OD for 4 weeks and their BP were evaluated, if their office DBP remained ≥ 90 mmHg after this period the dosage would be increased to 20 mg OD while the dosage of patients

whose office DBP were decreased to < 90 mmHg was adjusted down to 5 mg OD. The patients were administered with this new dosage regimen for another 4 weeks and their BP were again evaluated.

Office BP and 24-hour ABP were monitored after each treatment period.

*Procedures*

Office Blood Pressure measurement

Sitting SBP and DBP were measured with a mercury sphymomanometer (korotkoff I and V for SBP and DBP, respectively) on the left arm after subjects had been resting in the sitting position for 5 minutes (min). Three consecutive BP and heart rate(HR) were measured and recorded. All BP measurements were assessed in the morning just prior to the daily dose of enalapril.

24-hour ambulatory Blood Pressure measurement

24-hour ambulatory BP and HR were measured with a portable, non-invasive, fully automatic BP recordering machine (TM-2421; A&D company, Limited, Japan) which can be used alternatively between auscultatory and oscillometric methods.The adult cuff (range : 20-31 cm) was applied to the left arm of each subject and recordings were started shortly before the subjects took enalapril. The recorder was programmed to had at 30-min intervals for the entire 24-hour period. Subjects were allowed to have their normal daily activities after they left the hospital. However, they were instructed to remain motionless each time a reading was taken and to note down the times they went to bed and woke up in the morning.

*Data analysis*

*Office Blood Pressure measurement*

The average of the three BP and HR measurements was used for the evaluation. Sitting DBP was employed as an index of treatment response in that subjects who had a DBP reduction from baseline of ≥ 10 mmHg were defined as responders and those with a DBP at the end of the test treatment ≤ 90 mmHg were defined as normalized. The mean arterial pressure (MAP) was calculated as DBP plus 1/3 of the difference between SBP and DBP.

*24-hour ambulatory BP measurement*

BP was detected by oscillometric method, however in the case whose oscillometric measurement was failure the korotkoff BP will be used instead for the analysis. Raw data of ambulatory BP and HR were transferred to a computer programme. Systolic readings > 280 or < 60 mmHg, diastolic readings > 160 or < 40 mmHg, pulse pressure (SBP-DBP) > 150 or < 10 mmHg and pulse rate > 200 or < 35 beat per minute (bpm) were deleted. SBP, DBP, MAP, and HR values which were out of the range of mean ± 2 SD (standard deviation) of each parameter were also excluded.

Means of SBP, DBP, MAP and HR were calculated for each hour, the entire 24-hour, during day-time period (6.00 am-10.00 pm) and during night-time period (10.00 pm-06.00 am). Mean hourly values were derived from the average of 2 readings obtained in each hour. Forexample, the values at 8.00 and 8.30 were used for the calculation of BP value at 8 o'clock, day-time and night-time periods were defined as the time during awake and the time during sleep of each subject, respectively.

BP difference during sleep and awake was determined by subtraction of the mean or the average night-time BP from that of the day-time BP. Percentage of the reduction relative to the average day-time BP was also calculated.

**Dippers and Non-dippers**

Dippers were defined as those patients who had the reduction in night-time SBP and DBP > 10 % of their day-time values. Those whose night-time BP were not reduced by more than 10 % were defined as non-dippers.

**BP loads**

BP loads were BP values that were higher than 140 or 120 mmHg for SBP during day-time and night-time, respectively and 90 or 80 mmHg for DBP. BP loads were expressed both as the frequency or percentage and the absolute value of blood pressure (mmHg).

**Area under the blood pressure curve (AUC)**

AUC was calculated by using area under the systolic or diastolic BP curve, with SBP cutoff values of 140 mmHg during day-time and 120 mmHg during night-time and DBP cutoff values of 90 mmHg during day-time and 80 mmHg during night-time.

**Trough to peak ratio (T:P ratio)**

T:P ratio is the ratio between the antihypertensive effect at the end of the dosing interval (trough) and at the time of its maximum effect(peak). For each 24-hour ABP recording, trough SBP and DBP effects were BP reductions achieved between 23 and 24 hours after the dose, while peak SBP and DBP effects were the values averaged from the 2 adjacent hours giving maximum BP reduction which usually occur during 2-6 hours after the dose. T:P ratios were presented both as the

mean of each individual T:P ratio and as the T:P ratio obtained from using the mean trough and the mean peak values from all patients participated in the study.

**Antihypertensive response rate**

Antihypertensive response rate defined as BP reduction(mmHg) divided by administrative dose(mg)

*Statistical analysis*

- Result are presented as mean ± SD

- The OBP, 24-hour ABP measurements before and after treatments with 5, 10 or 20 mg enalapril OD were compared by using repeated measures ANOVA

- Compared antihypertensive effect of enalapril at the same dose on mild and moderate hypertensive patients by unpaired t-test

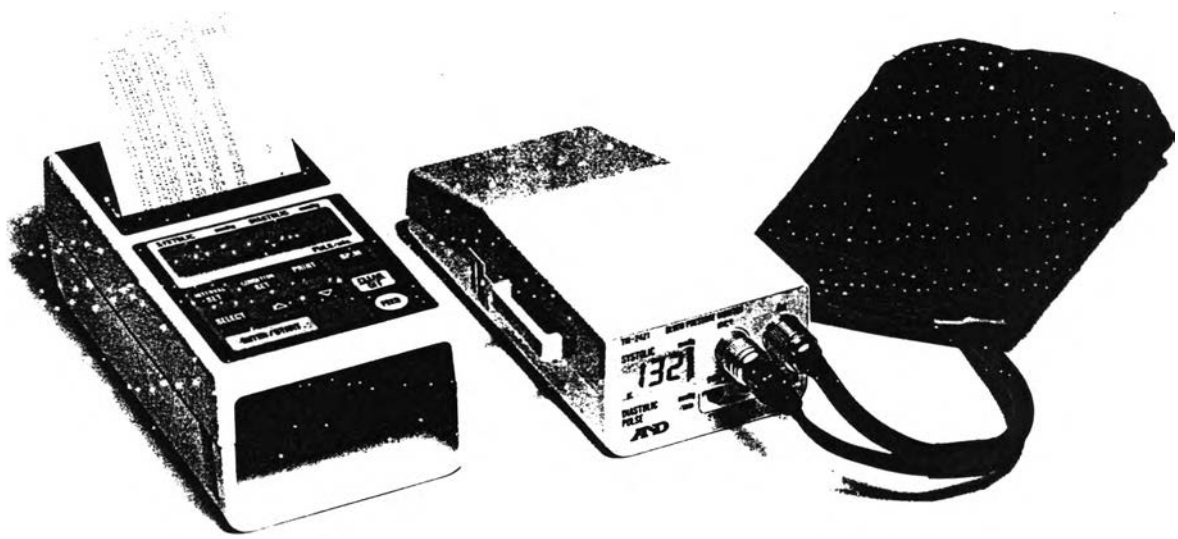
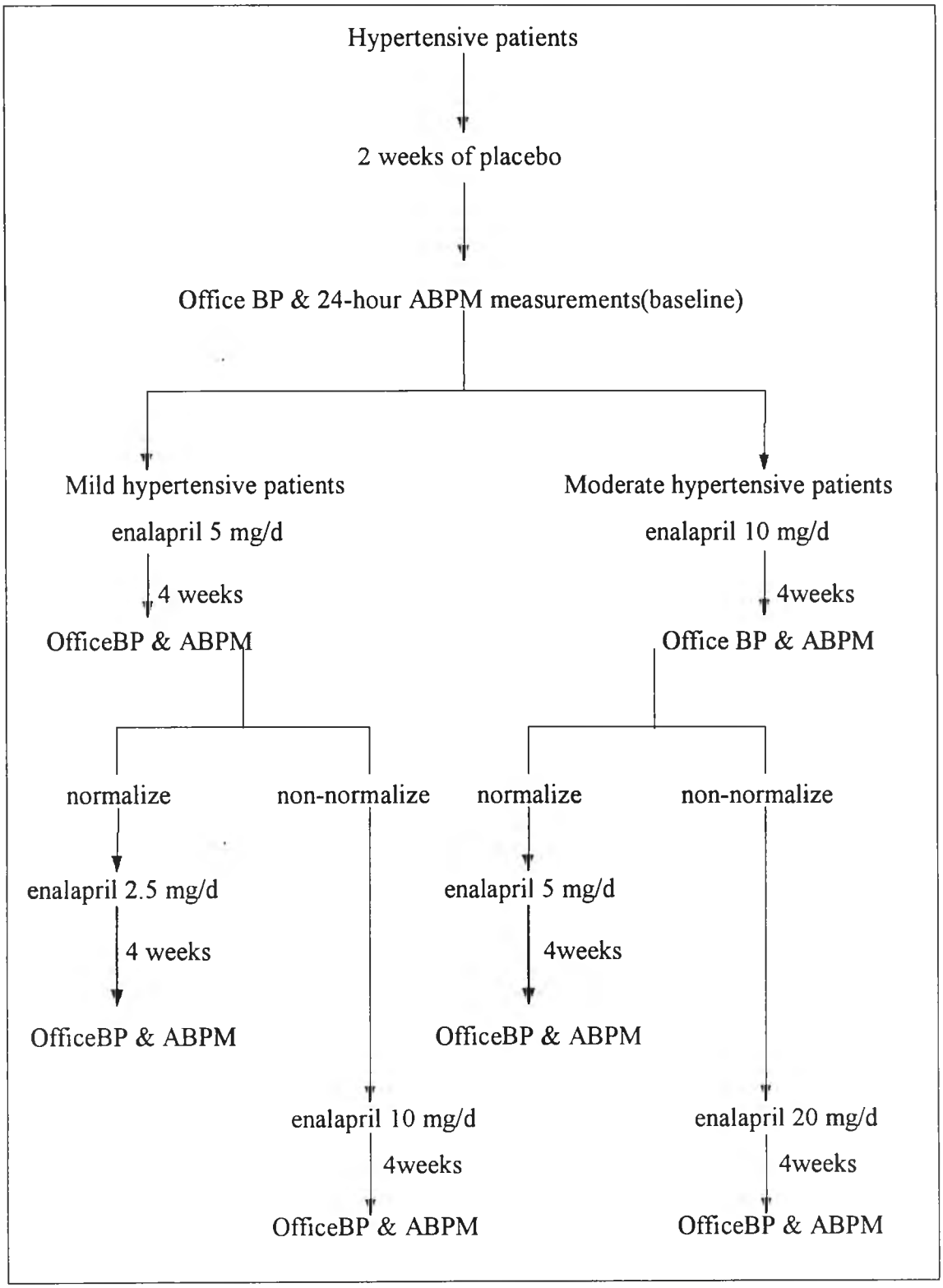



**Figure 3 : Ambulatory Blood Pressure Monitoring machine**




Note :- normalize in this study mean office SBP < 140 and DBP < 90 mmHg
non-normalize mean office SBP ≥ 140 but DBP < 90 mmHg or SBP < 140 but DBP ≥ 90 mmHg

**Figure 4 :** The study flow chart