CHAPTER IV

RESEARCH METHODOLOGY

4.1 Research Design : This is a multi-site, stratified, randomized, observer-blinded, parallel-group, controlled trial.

4.1.1 Research Design Model

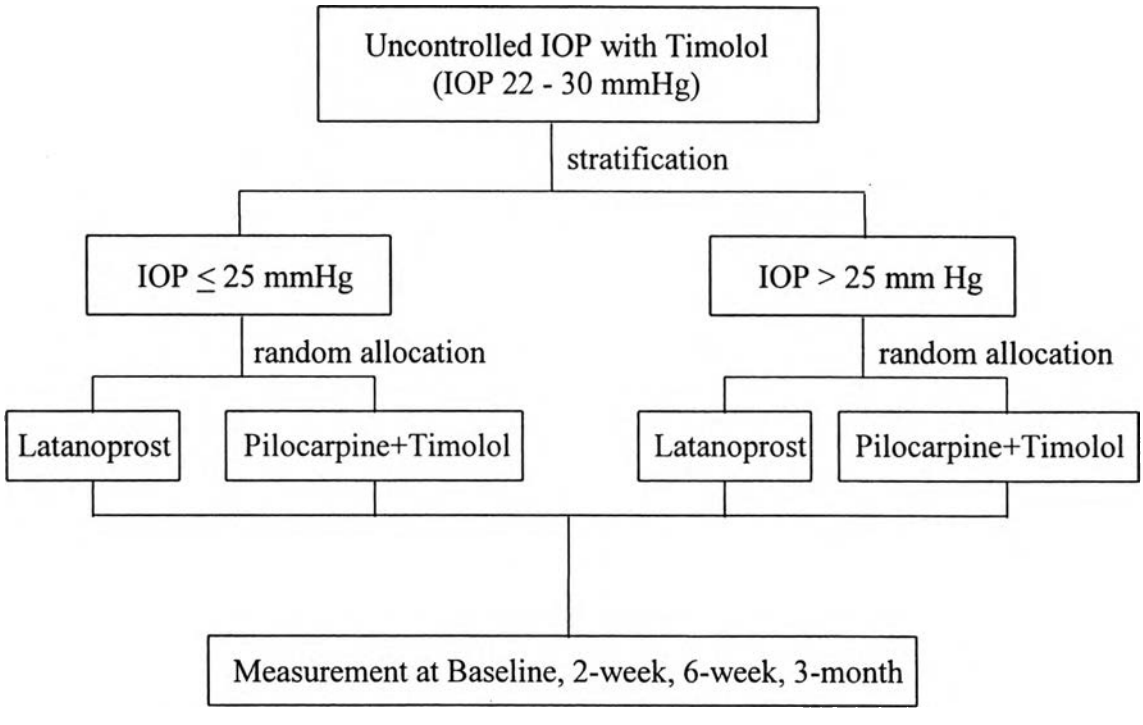

Fig. 4.1.1 Research design model

4.1.2 Management of Multi-site Study

This was a multi-site study conducted in 5 hospitals in Bangkok, Thailand. All of them are tertiary care hospitals. Multi-site study differs from multi-center study. Both types of study are conducted at more than one center and perform the same procedure on the same protocol. In multi-site studies, the investigators at the site do not participate as co-investigators of the study; they are merely carrying out the study (e.g. recruiting subjects, treating subjects and following subjects). But in multi-center study, the investigators at the site are involved as co-investigators in the planning of the study protocol and procedures, are responsible for the study results, and participate in manuscripts and other dissemination activities.

Even when the study is a multi-site study, it is important to ensure that all centers follow the study protocol. Thus, firstly we arranged a meeting of investigators from all centers at the planning stage to obtain agreement prior to starting the study.

Secondly, for quality control in measurement and clinical observation, we explicited the detail of outcome measurements and did intra-observer and inter-observer reliability test before the trial began.

Thirdly, for data recording, collection and processing of data; since this was a small to moderate size study, I, as a principal

investigator (PI) took a responsibility as a data manager to receive data and provide feedback to participating center.

Fourthly, to motivate all participants to play an enthusiastic and responsible role, I, as the PI made a phone call to all investigators monthly to keep up with the activities of the study.

4.2 The Sample

4.2.1 Target population : Adult patients with open-angle glaucoma or ocular hypertension who are inadequately controlled with timolol.

4.2.2 Sample population : Adult patients with open-angle glaucoma or ocular hypertension who are inadequately controlled with timolol, come to glaucoma clinic, at one of the 5 centers that participates in this study and who meet the following eligibility criteria.

4.2.3 Eligibility Criteria

4.2.3.1 Inclusion criteria

1. Patients are older than 18 years of age.

2. Diagnosed to have unilateral or bilateral primary open-angle glaucoma, pseudoexfoliation glaucoma, pigmentary glaucoma or ocular hypertension by ophthalmologists at any of the 5 study centers.

3. Have received monotherapy with topical beta-blockers (timolol) with inadequate IOP control. (IOP = 22 - 30 mmHg in any eye)

4. Have received any two topical antiglaucoma drugs (except prostaglandin derivatives) that after stopping the drugs and run in with 0.5 % timolol twice a day for two weeks, the patients still have IOP = 22 - 30 mmHg in any eye.

5. Subjects have best corrected visual acuity of 20/100 or better in each eye.

6. Patients requiring bilateral treatment have to fulfill all eligibility criteria for both eyes to be included. If only one eye fulfills the inclusion criteria, however, that eye is included in the study as a study eye but the other eye can be treated with allocated study therapy provided that no exclusion criteria are met.

4.2.3.2 Exclusion criteria

1. Patients with only one sighted eye or amblyopia

2. Closed anterior chamber angle, severe ocular trauma at any time, current use of contact lens, ocular inflammation or infection within the last 3 months

3. Corneal abnormality that prevents reliable applanation tonometry

4. Severe retinal disease, severe glaucomatous damage with a cup/disc ratio greater than 0.8, split fixation or clinically significant (in the investigator's opinion) field loss within the central 10 degrees, or legal blindness in either eye

5. Had intraocular surgery within the past 12 months, eye laser surgery within the past 3 months

6. Currently pregnant or nursing

7. Active ocular disease

8. Using any ophthalmic, dermatologic, or systemic corticosteroid

9. Known hypersensitivity to any ingredients in the study medication

10. Contraindications to β - blocker therapy

11. Concomitant use of systemic medications known to affect IOP was not allowed; however, oral beta blockers were allowed if the dosage remained constant throughout the study.

12. Any pseudophakic or aphakic eye will be excluded. But if the other eye can fulfill all eligibility criteria, that eye can be included in the study.

4.2.4 Sample Size Estimation

Since the primary outcome is the mean of IOP reduction, the sample size formula for comparing two mean differences of two independent groups was used.

$$\text{N/group} = \frac{2\,(Z_\alpha + Z_\beta)^2\,\delta^2}{(\bar{X}_1 - \bar{X}_2)^2}$$

Where $\alpha = 0.05$, $\beta = 0.1$ (power = 90%)

$Z_{\alpha/2} = 1.96$, $Z_\beta = 1.28$

$\delta = 3$ (from review literature[9-12])

$\bar{X}_1 - \bar{X}_2 = 2$ (extensive debate with content experts)

$\text{N/group} = 2\,[1.96 + 1.28]^2\,(3)^2 / (2)^2$

$= 189/4$

$= 47.25 = 48$ cases

Total sample size = 96 cases

To compensate for withdrawals and drop–outs (10%)

$N/(1 - r) = 96/0.9 = 106.6$

$= 107$ cases

Then total sample size of this study is 107 cases.

4.3 Experimental Maneuver

4.3.1 Randomization Method

In this study we used **stratified randomization**, using random permuted blocks within strata. The stratified randomization is designed to allocate patients to different treatments to achieve approximate balance of important characteristics, without sacrificing the advantages of random allocation. The details were:

1. In this study, the patients would be stratified into 2 strata; the patients whose IOP were between 22-25 mmHg and those whose IOP were between 25.1-30 mmHg.

2. After completing randomization list, (block of 4, using a table of random numbers, was applied within each stratum), the code was kept in sealed envelopes and distributed as estimated sample size to each center. Each center received two sets of envelop, the first was the set of IOP 22-25 mmHg, and the second was the set of IOP 25.1-30 mmHg.

3. When eligible patients registered to the trial, the investigator picked up the envelop from each set of specified IOP, as prepared. (If IOP in one eye was less than 25 mmHg and IOP in the other eye was more than 25 mmHg, the randomization list in less than 25 mmHg strata would be used).

4.3.2 Blinding Method

To avoid any biases in the comparison of the groups, the blinding method is desirable. In this study we used **observer blinded design,** while examining patients, the investigator did not know what group of treatment the patients were enrolled in. Because the primary outcome measurement (IOP) is considered to be a hard outcome, is directly measured by observers and highly reliable. If we had also wanted to blind the patients, all patients would have to instill the eye drop 6 times a day, that would be very inconvenient for them.

4.3.3 Intervention

This is a multi-site study, conducted in 5 hospitals in Bangkok. The protocol was approved by the Institutional Review Board of each hospital.

The procedure in this study were:

1. One or two well trained ophthalmologists in each center conducted the study.

2. The investigator assessed the patients who fulfilled the eligible criteria.

3. Informed consent was signed after proper counseling and describing the detail of the study including side effects of the drugs by one of the investigators or specified assistant.

4. Each patient received 0.5% timolol (Timoptol ® MSD) eye drop twice daily for a 2-week-run-in period.

5. To enter the study, baseline IOP was required to be 22 mmHg or more and less than 30 mmHg in any eye at the end of the run-in period.

6. After that, patients were atratified into 2 strata ; the first stratum was IOP $\leq$ 25 mm Hg., the second stratum was IOP > 25 mmHg, and randomized to two paralled study groups.

7. The first group received monotherapy of latanoprost 0.005% eye drop once daily (at 8.00 PM), the second group received combination therapy of pilocarpine 2% eye drop four times daily (at 7.00 AM, 11.00 AM, 4.00 PM and 8.05 PM) and timolol 0.5 % eye drop twice daily (at 8.00 AM, and 8.00 PM).

8. Patients were explained about dosage and method of drug application, side effects of drugs, importance of adhering to protocol, and schedule of examination.

9. Examination was performed at baseline, 2-week, 6-week and 12-week follow up. The schedule of examinations and procedures is presented in Table 4.3.3.

10. Patients who were judged by the investigator to require additional therapy to control IOP, after randomization, would be discontinued from the study.

TABLE 4.3.3 Schedule of examinations and procedures

	Within 4 weeks of	Baseline			2 weeks	6weeks	12 weeks		
Examination	Baseline	9.00 AM	12.00 PM	3.00 PM	9.00AM	9.00 AM	9.00 AM	12.00 PM	3.00PM
- Medical and ocular history	X								
- Gonioscopy	X								
- Visual fields	X						X		
- Ophthalmoscopy	X						X		
- Symptom	X	X			X	X	X		
- Visual acuity	X	X			X	X	X		
- Refraction	X						X		
- Slit – lamp examination	X	X			X	X	X		
- Intraocular pressure	X	X	X	X	X	X	X	X	X

4.3.3.1 To get good compliance: The dosage and method of drug application were explained to every patient. The detail and importance of patient cooperation were emphasized at the beginning of the study and at every visit. Patients were asked to bring their eye drop bottles to the physicians at each visit for estimating their weight.

4.3.3.2 Avoidance of cointervention and contamination: The patients were asked not to use any eye drops or medication except what they got from the study. This was repeated at every visit. However, these drugs are not commonly prescribed by drug stores or by other personnel except ophthalmologists.

4.4 Measurement

Variables : Independent Variable = Intervention given

: Dependent Variable = IOP reduction

4.4.1 Instruments and Evaluators

Medical and ocular history, gonioscopy, perimetry, ophthalmoscopy, symptomatology, visual acuity, refraction, slit–lamp examination, and IOP measurement were performed according to the schedule presented in Table 4.3.3. During the 12- week study period, there were four scheduled follow-ups, at baseline, 2 weeks, 6 weeks and 12 weeks. Intraocular pressure was measured in mmHg using Goldmann applanation tonometer affixed to a slit lamp. The patients were well prepared in sitting position, relaxed and comfortable, with slightly extended neck, and not wearing tight clothing. Their eyes were examined in primary position (looked straight ahead). The applanation tonometer should be checked and calibrated at least once a year, preferably twice a

year, following the techniques indicated in a tonometer's operator's manual.

4.4.2 Outcome To Be Measured

1. Main outcome: The primary outcome is IOP reduction from baseline, measured in mmHg. Two measurements were performed on each eye, and the mean of that two measurements is used in the statistical analysis. Diurnal IOP was defined as the mean value of measurements taken at 9.00 AM, 12.00 PM and 3.00 PM. If both eyes of a patient were studied, the average IOP of that both eyes would be used .

2. Secondary outcome

2.1 The success rate of treatment (number of patients who reached target IOP≤15, ≤18 and ≤ 21mmHg) in each treatment group would be analyzed.

2.2 The response rate of treatment (number of patients whose IOP reduction from baseline ≥ 10%,≥ 20%, ≥ 30% and ≥ 40%) in each treatment group would be analyzed.

2.3 Ocular and systemic side effects of both groups would be detected and compared. (Table 4.6.1.2)

2.4 Cost-effectiveness of both groups would be evaluated.

4.5 Data Collection

As this is a multi-site study, all forms were prepared and collected at the trial coordinating center (Ophthalmology Department, Bhumipol Adulyadej Hospital). A principal investigator also acted as a data manager whose duty was:

1. Distributed every form to each investigator before the trial started.

2. Got all trial data in good shape ready for statistical analysis. We had a folder for each patient's record, being ordered by trial number.

3. Carried out a series of checks : general checks, missing data checks, range checks and logical checks.

4. Any problem identified by these checks were conveyed back to the study site so that corrections were made.

5. Actively seeking forms from the study site when they were overdue.

4.6 Data Analysis

4.6.1 Summarization of Data

For continuous data such as age and IOP: the mean, SD, and range were analyzed. For categorical data such as sex, type of glaucoma and side effects: number and/or percentage were presented and analyzed as appropriate. (Table 4.6.1.1 and Table 4.6.1.2)

Table 4.6.1.1 Statistical analysis for demographic data

Variables	Type of data	Data summary
Baseline and demographic data		
- Age	Continuous	Mean, SD, range
- Sex	Categorical	Number, percent
- type of glaucoma	Categorical	Number, percent
- Baseline IOP	Continuous	Mean, SD, range

Table 4.6.1.2 Statistical analysis for outcome variables

Variables	Type of data	Data summary	Statistical test
Primary Outcome - IOP reduction from baseline	Continuous	Mean, SD	3 way ANOVA
Secondary Outcome			
- Headache, browache (0,1)	Categorical	number	Fisher's exact test
- Eye discomfort, eye irritation (0,1)	Categorical	number	Fisher's exact test
- Decrease vision(0,1)	Categorical	number	Fisher's exact test
- Superficial punctate keratitis(0,1)	Categorical	number	Fisher's exact test
- Follicular conjunctivitis(0,1)	Categorical	number	Fisher's exact test
- Presence of cell/flare (0,1,2,3)	Categorical	number	Fisher's exact test
- Conjunctival hyperemia (0,1)	Categorical	number	Fisher's exact test
- Changes of eyelashes (0,1)	Categorical	number	Fisher's exact test
- Iris hyperpigmentation (0,1)	Categorical	number	Fisher's exact test
- Cystoid macular edema(0,1)	Categorical	number	Fisher's exact test
- Cup/disc ratio change (0,1)	Categorical	number	Fisher's exact test
- Visual field change (0,1)	Categorical	number	Fisher's exact test
- Visual acuity change (0,1)	Categorical	number	Fisher's exact test
- Blood pressure (mmHg)	Continuous	Mean, SD	3 way ANCOVA
- Heart rate (pulse/min)	Continuous	Mean, SD	3 way ANCOVA
- Response rate of both groups (IOP reduction≥10, ≥20, ≥30, ≥40%)	Categorical	number (%)	Fisher's exact test
- Success rate of treatment (IOP≤15, ≤18 and ≤21 mmHg)	Categorical	number (%)	Fisher's exact test

4.6.2 Data Presentation

The table, graph and bar chart would be presented as appropriate.

4.6.3 Hypothesis Testing

This study was done to compare two mean differences of two independent groups. The primary effectiveness end point was IOP reduction from baseline. The **primary effectiveness analysis** was based on an intention to treat analysis, patients were analyzed according to their randomized treatment, irrespective of whether they actually received the treatment, with last observation carry forward approach.

To validate the primary analysis, a **secondary analysis** would be performed using the Per-Protocol-Observe Cases approach in which examinations associated with a serious violation of the protocol would be excluded and missing data points were not estimated.

Histogram, normal P-P Plot of mean IOP at baseline and final visit of both treatment groups and test for normal distribution of data with One-Sample Kolmogorov-Smirnov test, using SPSS 9.01 for windows were done first.(Appendix E-I) Then the difference in diurnal IOP reduction between the two treatment groups was determined using 3 way ANOVA : the model used mean IOP at final visit as dependent

variable, used study drug, stratified patients (into 2 groups, baseline IOP ≤ 25 and > 25 mmHg) and center as fixed factors.

Secondary outcomes were reported in number and/or percentage and analyzed as appropriate.

4.6.4 Problem from Protocol Deviation: Deviations from randomized allocation often result in missing outcome data. Full report of any deviations from random allocation and missing response are essential in the assessment of intention to treat approach. Various imputation methods may be used to estimate the missing responses, in this study we used the last observed response (carry forward) for the analysis.

4.6.5 Cost-effectiveness Analysis: In this paper only direct medical cost (drug cost) and effectiveness of the drug were analyzed. The main outcome measurement in this cost-effectiveness analysis was number of patients who have IOP$\leq$15, $\leq$18 and $\leq$21 mmHg. The drug cost/year/100 patients and the effectiveness/100 patients for both groups were calculated.

Cost-effectiveness ratio (Baht/one patient IOP control/year) for each group would be analyzed by using:

$$\text{Cost-effectiveness ratio} = \frac{\text{cost/year/100 patients}}{\text{effectiveness/100 patients}}$$

Incremental analysis would be performed by using:

$$\text{Incremental CE ratio} = \frac{\text{cost A - cost B}}{\text{effectiveness A - effectiveness B}}$$

The result will show how much money we have to pay if we want to cure (IOP control) one more patient by changing the drug from drug B to drug A.

Sensitivity analysis: varying cost of latanoprost and varying cost of timolol would be done.

4.7 Ethical Consideration

1. The study was approved by Institutional Review Board of each hospital.

2. The patients were given all information about the study, both in the trial and in the alternative treatment, together with side effects and

potential adverse effects including consequences. After they agreed to participate, they had to sign an informed consent.

3. From the literature review, latanoprost is a new drug which is highly effective in lowering IOP in glaucoma patient, with low ocular and systemic side effects. Thus the intervention could provide more benefit than harm.

4. Patients who were judged by the investigator to require additional therapy to control IOP, after randomization, were discontinued from the study.

5. Withdrawal from the study did not interfere with regular care or benefit of the patients.

6. The data was kept confidentially.

4.8 Limitations

There may be not enough sample size, patients with open-angle glaucoma and ocular hypertension, who fulfill the eligibility criteria, uncontrolled IOP with timolol treatment in the limited time of the study. There are about fifty patients in glaucoma clinic, Bhumibol Adulyadej Hospital, but they are fewer than half of the patients that fit to this study. Multi-site study is the solution of this problem.

4.9 Benefits of the Study

In many patients, topical beta–adrenergic antagonists alone do not sufficiently lower IOP, and additional medications have to be prescribed. However, administration of several medications may be inconvenient for patients. Latanoprost is used once daily with high efficacy. Hence, long term maintenance with latanoprost monotherapy would be advantageous for patients, not only for improving quality of life but also for good compliance. Besides, there is little evidence about latanoprost study in Thai people. So if the study reveals very promising result in Thai people, the application of this drug will be very beneficial to them. Apart from that, cost-effectiveness analysis will help clinician in decision making for preseribing drugs for their patients.

4.10 Obstacle

Blinding technique: To avoid any biases in the comparison of the groups, the blinding method is desirable. In this study we used observer-blinded design because if we had wanted to blind the patients also, all patients would have to instill the eye drops 6 times a day, that would be very inconvenient for them. (The patients in latanoprost group used the eye drop once daily.) In this study, using a blinded evaluator should be sufficient to reduce bias in treatment comparison because our

primary outcome is IOP, which is considered to be hard and objective outcome, and is directly measured by the observer.

4.11 Administration and Time Schedule

Preparation	.....February – March 2000
Training of personnel	.....March 2000
Data collection	.....April 2000– June 2001
Data analysis	.....July 2001
Thesis writing	.....August 2001
Presentation	.....September 2001