# CHAPTER III

# 3. RESEARCH DESIGN AND METHODOLOGY

## 3.1 Research Questions

### 3.1.1 Primary Research Question

What are the normal values of topographic optic disc parameter measurements in the normal Thai population using the Heidelberg Retina Tomograph?

### 3.1.2 Secondary Research Question

Are there any relationships between age and optic disc topography?

## 3.2 Research Objectives

### 3.2.1 Primary Objective

To establish normal values of topographic optic disc parameters in Thai population.

### 3.2.2 Secondary Objectives

To explore the relationship of age on optic disc topography.

## 3.3 Hypothesis : (none)

## 3.4 Conceptual Framework

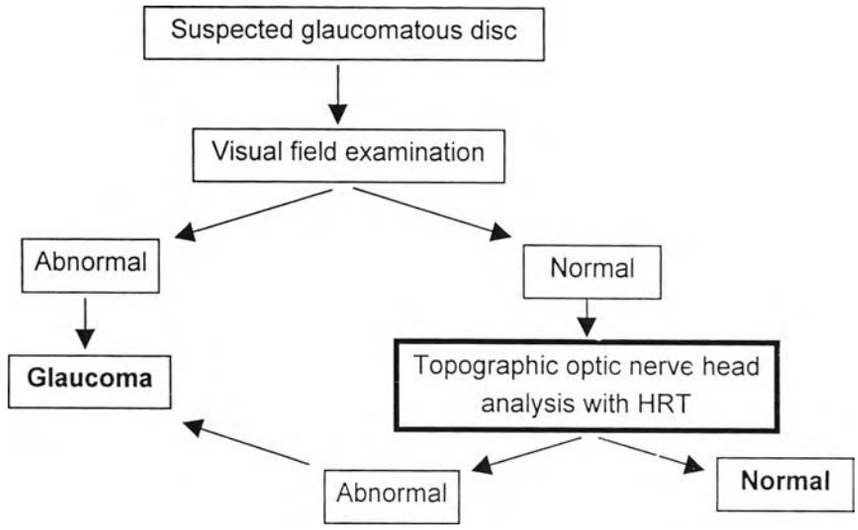
Glaucoma is a group of diseases characterized by damage of the optic nerve head (optic disc) and specific patterns of visual fields loss. Increased intraocular pressure is a significant risk factor for developing glaucoma but it is not a cause. Classically, the gold standard of glaucoma diagnosis is based on evidence of typical visual field defects from a visual field test that correspond to the optic nerve head damage. Recent development of imaging technique, particularly the Heidelberg Retina Tomograph (HRT), has shown that it is possible to detect damages in the optic nerve head from glaucoma years before visual field defects occurred. Unfortunately, HRT is still a new technology and is not widely used in the clinical practice. Visual field testing is still a routine procedure in most of the clinics.

Early diagnosis of glaucoma by the HRT required a normal database of optic disc topography. The present normal data in the HRT is from non-Thai population. As mentioned earlier, optic disc topography can be different in different ethnic groups. It is obvious that we need a Thai specific normative data of optic disc topography in order to use the HRT with Thai patients.

The following diagram (figure 3.1) illustrates the concept of diagnosing a patient with glaucoma suspect. The present routine is to send the patient for visual field examination. The doctor will diagnose glaucoma only when the patient has

definite glaucomatous visual field defect. However, the patient with normal visual field but has abnormal optic disc topography by HRT will also be diagnosed as glaucoma according to the new concept. Patients with abnormal HRT will have visual field defects years after.

**Figure 3.1** Conceptual Framework




## 3.5 Assumptions : (none)

## 3.6 Key Words

Optic disc topography, confocal scanning laser ophthalmoscope, HRT, normal value.

## 3.7 Operational Definitions

### 3.7.1 Definition of Thai

1) Persons whose one or both of their parents have Thai nationality.
2) The subjects can be a mixture of Thai-Chinese, Thai-Lao, Thai-Vietnamese etc.
3) Persons whose one of their parents have other racial mixture such as Caucasian, European or African-American ethnicity will not be considered as Thai persons.
4) The confirmation of the nationality will be by interview (see inclusion criteria below).

### 3.7.2 Definition of Normal Eye

1) Intraocular pressure less than 21 mmHg
2) Normal visual field; has visual field score of 0 according to the Advanced Glaucoma Intervention Study (AGIS) criteria[54] (see appendix A)
3) Best corrected visual acuity of 20/40 or better
4) Refractive error of <+3 D and <-6 D, astigmatism < 2.5 D
5) No history of ocular trauma
6) No history of ocular surgery other than cataract extraction

7) No family history of glaucoma in a first-degree relative

8) No ophthalmic diseases that could affect the optic nerve e.g., ischemic optic neuropathy, retinal vascular occlusion.

9) No underlying systemic diseases that could affect the optic nerve e.g., diabetes mellitus, hypertension.

## 3.8 Research Design

Crossectional descriptive study

### 3.8.1 Target Population

Thai people in the Northeast age 30-60 years old.

### 3.8.2 Sample Population

Thai people age 30-60 years old who lived in Ubolrat district in Khon Kaen province during the year 2000-2001 which fulfill the following inclusion criteria.

### 3.8.3 Inclusion Criteria

1) Thai persons age 30-60 years old (see definition of Thai above and remark below)

2) Normal eye by the definition above

**Remark:** Thai nationality was confirmed by the following steps.

1) The subject was asked for an identification (I.D.) card. Only Thai person could have an ID card.

2) The health workers (from Ubolrat hospital) then interviewed the subject for the nationality of his parents and grandparents. If there was any doubt, the I.D. and house registration of their parents and grandparents were checked.

3) If the I.D. and/or house registration were not accessible, neighbors and/or headman of the village were interviewed to confirm the subject's nationality.

4) Finally, if there was still any doubt, the subject was excluded and a new subject was randomly selected.

### 3.8.4 Exclusion Criteria

1) Any pathologic ocular conditions that preclude pupil dilatation.
2) Allergic to Tropicamide or topical anesthetics.
3) Occludable angle (preclude pupil dilatation)
4) The subjects could not sit still for examination.

**Remark:** Ubolrat district was chosen for the study from the following reasons:

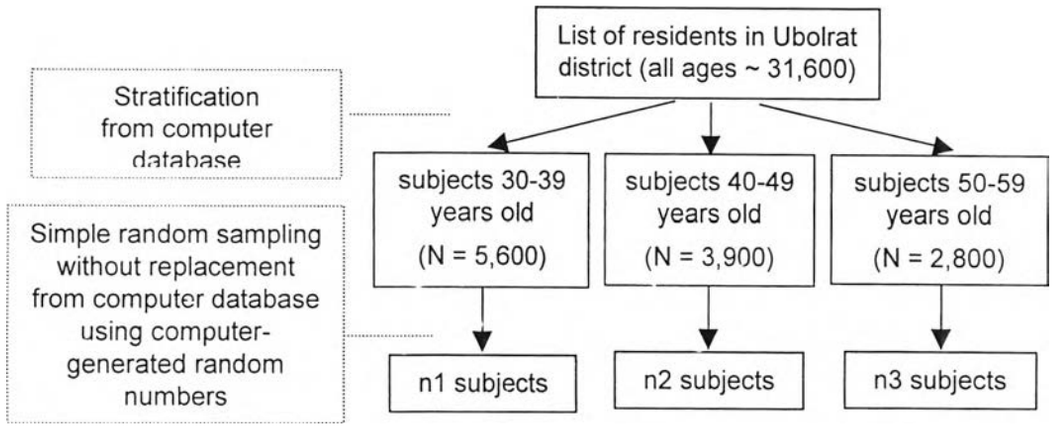
1) It is an average size rural city (population about 30,000)
2) It is not very far from the city of Khon Kaen (50 km).
3) The people have average socioeconomic status.
4) The people in Ubolrat have the same culture and habits as the rest of people in the northeast.
5) Complete demographic database is available from Ubolrat Hospital computer.

### 3.8.5 Sampling Technique

Since there is a rather complete (>90%) database of population in Ubolrat district stored in the Ubolrat's hospital computer, a stratified simple random sampling technique was used. We first stratified the subjects (using data from the computer) into three age groups; 30-39, 40-49 and 50-59 years old. Then simple random sampling (without replacement) from each group was done by means of computer generated random number. The diagram below (figure 3.2) illustrates the sampling process.

To ensure that we have the most variation of the subjects, subject in the same family will be discarded. In other words, we will collect only one subject from each family. The family will be checked by last name in the database and by history taking.

**Figure 3.2** Diagram showing the sampling process.




### 3.8.6 Sample size calculation

Since there was no data on optic disc topography in Thai population the sample size was estimated based on the previous published data. The data from Tsai et al[33] had the descriptive statistics of some optic disc parameters obtained with HRT in Asian normal subjects (Table 3.1) and would be appropriate for Thai population. The data from Tsai did not have the data for each separate age group. To stay on the safe side, we will use the variance of the whole group to calculate sample size for each age group.

The calculation used the confidence level and the precision that the investigator specified to calculate the sample size. We set the confidence level at 95% and set the precision (half the total width of the confidence interval) which we thought were relevant clinically as summarized in Table 3.1.

To calculate the sample size the formula below was used. [55]

$$n = \frac{Nz^2s^2}{d^2(N-1) + z^2s^2}$$

Where n = number of samples
N = the size of the sampling population
z = critical value, for 95% confidence level ($\alpha$= 0.05) and two-sided interval, z = 1.96
s = standard deviation
d = precision or desired half-width of the confidence interval (W/2)

This formula take into account the finite population correction factor which is sqrt {(N – n) / (N - 1)}.

The population of Ubolrat district that was registered in the computer database is about 31,600 (as of January 2000). The population in each decade from 30 to 60 years old was calculated to be 5600, 3900 and 2800 respectively. These numbers were the sampling frame N (of each age group) for sample size calculation. Since N was rather large, the finite population correction in the above formula had little effect on the result and can be ignored. The sample size for each age group, calculated based on the data (mean, sd) from Tsai, is presented in Table 3.2.

**Table 3.1** Optic disc topography of 45 Asian normal subjects (age 19-40) obtained from HRT

| | Mean | SD | 95% CI | Width of interval | Desired width of interval (W) | Precision (d) |
|---|---|---|---|---|---|---|
| **Disc area ($mm^2$)** | 2.61 | 0.48 | 2.47 to 2.75 | 0.28 | 0.20 | 0.100 |
| **Cup area ($mm^2$)** | 0.53 | 0.48 | 0.39 to 0.66 | 0.27 | 0.20 | 0.100 |
| **Rim area ($mm^2$)** | 2.08 | 0.34 | 1.99 to 2.18 | 0.19 | 0.15 | 0.075 |
| **Rim volume ($mm^3$)** | 0.65 | 0.24 | 0.59 to 0.72 | 0.13 | 0.10 | 0.050 |

**Table 3.2** The calculated sample size of each age group

| | SD | Precision (d) | 30-39 year old (N=5,600) | 40-49 year old (N=3,900) | 50-59 year old (N=2,800) |
|---|---|---|---|---|---|
| **Disc area ($mm^2$)** | 0.48 | 0.100 | 88 | 87 | 86 |
| **Cup area ($mm^2$)** | 0.48 | 0.100 | 88 | 87 | 86 |
| **Rim area ($mm^2$)** | 0.34 | 0.075 | 78 | 78 | 77 |
| **Rim volume ($mm^3$)** | 0.24 | 0.050 | 88 | 87 | 86 |

From Table 3.2 above, the calculated sample size was about 90 for each age group.

## 3.9 Data Collection

Questionnaire form was used for history taking. All of the optic disc topography data was stored in digital format in the hard drive. Data collection form was used to summarize the topographic data from each subject (Appendix E). The following data was collected.

### 3.9.1 Administrative and Demographic data

Identification number
Name
Address
Age
Sex
Ethnicity

### 3.9.2 Baseline data

Visual acuity
Refractive error

Corneal curvature (k)
Intraocular pressure
Visual field indices

### 3.9.3 Data computed by HRT software

HRT software version 2.01 was used to calculate 12 topographic optic disc parameters after each scan. These were all continuous variables. The following table (Table 3.3) summarized the names used for these parameters in the analysis and their units.

**Table 3.3** Twelve topographic optic disc parameters (HRT parameters), their variable names and their units.

| HRT Parameters | Variable name | Unit |
|---|---|---|
| **disc area** | diskarea | $mm^2$ |
| **cup area** | cuparea | $mm^2$ |
| **cup-to-disc area ratio** | cdaratio | None |
| **rim area** | rimarea | $mm^2$ |
| **cup volume** | cupvol | $mm^3$ |
| **rim volume** | rimvol | $mm^3$ |
| **mean cup depth** | meancup | mm |
| **maximum cup depth** | maxcup | mm |
| **cup shape measure** | cupshape | None |
| **height variation contour** | hvcontou | mm |
| **mean RNFL thickness** | meanrnfl | mm |
| **mean RNFL cross section area** | rnflarea | $mm^2$ |

### 3.9.4 Ophthalmic examination

All subjects were transported and examined at Srinagarind Hospital in the ophthalmic outpatient unit. To ensure that the subjects did not have glaucoma and other eye diseases, the following examinations were done:

1) History taking
2) Visual acuity with Snellen chart
3) Refraction, corneal curvature with an autorefractor
4) Visual field test with a computerized visual field instrument, the Humphrey Field Analyzer using full threshold program 24-2.
5) Intraocular pressure (IOP) by a standard Goldmann applanation tonometer.
6) Slit-lamp examination

After slit-lamp examination the pupils of subjects were dilated with 1 drop of 1% tropicamide. If after 15 minutes the pupil was smaller than 4 mm, another drop of 1% tropicamide was administered. When the pupil dilated to 4 mm or more the subjects was examined with an indirect ophthalmoscope, HRT and disc photograph.

7) Dilated fundus examination using an indirect ophthalmoscope.
8) Detailed disc examination using a slit-lamp and special lenses.

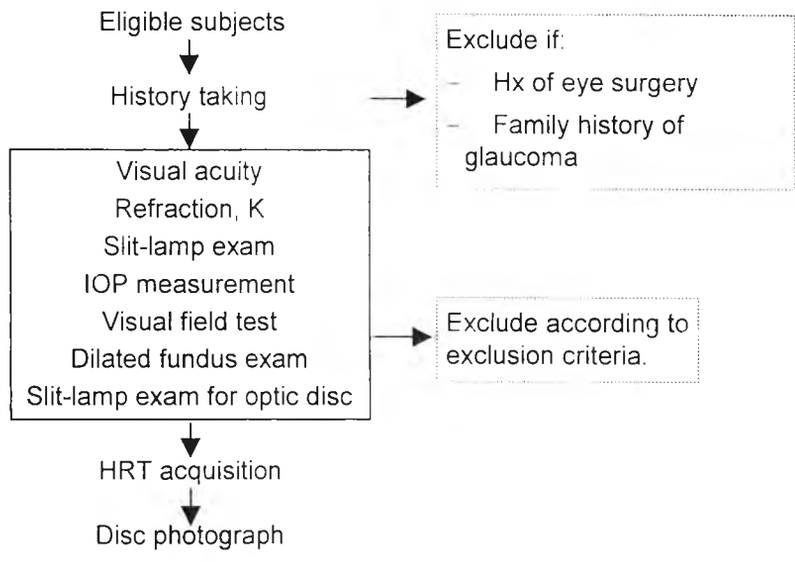
9) HRT image acquisition
10) Disc photograph

### 3.9.5 HRT Image Acquisition

HRT image acquisition was done after pupil dilatation with field of view of 10 x 10 degrees. Three images for each eye was scanned to obtained a mean topography image. This procedure reduced the variability of the measurements as recommended by Weinreb.[10] After image acquisition, a contour line was drawn around the optic disc and then the 12 topographic optic disc parameters was calculated with the HRT software using the standard reference plane.

The following diagram summarized the sequence of procedures in the examination (figure 3.3).

**Figure 3.3** Diagram showing the examination sequence.




## 3.10 Data analysis

### 3.10.1 Baseline data (age and sex)

Percentage of male and female subjects for each age group was summarized and geographic distribution of the subjects was presented in tables.

### 3.10.2 Unit of Analysis for HRT parameters

For subjects that had data from only one eye (one-eyed subjects), that eye was used for statistical analysis. For subjects that had data from both eyes (two-eyed subjects), the average of the two eyes was used for statistical analysis.

### 3.10.3 Description of the HRT Parameters

The mean values, standard deviation and confidence interval were presented. The 2.5$^{th}$ (P2.5), 25$^{th}$ (P25), 50$^{th}$ (P50 or median), 75$^{th}$ (P75) and 97.5$^{th}$ (P97.5) percentiles of the 12 HRT parameters were calculated. The calculations of these values were not straightforward because our data was not simple random

sampling from the whole population. We first stratified the population into 3 age groups and then simple random sampling in each age group to get the subjects (which were the primary sampling units or PSUs). These design effects will have a substantial influence on the results of variance estimates and standard error of the population mean. The design effect (deff) is defined as

$$deff = V / V_{srswor}$$

where V is the design-based estimate of variance (which take into account the sampling weights and stratification) and $V_{srswor}$ is an estimate of what the variance would be if a similar survey was conducted using simple random sampling (srs) without replacement (wor) with the same number of sample elements as in the actual survey.

These survey-design features: probability sampling weights and stratification were taken into account for all the calculations. With this approach, the variance estimates were produced using Taylor-series linearization methods. Finite-population corrections for simple random sampling without replacement of PSUs were computed. This method will usually give more precise estimates and narrower confidence intervals.

The distributions of the HRT parameters were explored by graphing histogram, dot plot, outlier box plot, standardized normal probability plot (p-p plot), quantile normal plot (q-q plot) and symmetry plot. Normality of the distribution were checked by normal plots and the Shapiro-Wilk W test. This test is preferred to the Kolmogorov-Smirnov test for normality because the mean and standard deviation of the normal distribution are not known for the HRT parameters.

### 3.10.4 Reference Intervals (Normal Range)

The ultimate goal of this study is to establish normal range for the topographic optic nerve parameters (HRT parameters). In reality this is not quite easy and straightforward due to the definition of "normal range". For example, in the UK population almost everyone has hard fatty deposits in their coronary arteries, which result in death for many of them. In the contrary, very few Africans have this. We usually say that normal people are the apparently healthy members of the local population.[56] The next problem is to estimate the set of values for this "normal range". One common statistical procedure is to leave 5% of "normals" outside the "normal range" which sounds contradictory: no matter how medically "normal" the people may have been, 5% of them must emerge as "abnormal" after the statistical partitions.[57] This contradiction comes from the different meaning of "normal" in medicine and "normal distribution" in statistics. To avoid this confusion the terms "95% reference range" or "95% reference interval" had been used and becoming widely accepted.[56] In this study we will use the term "95% reference interval" (95%RI) instead of normal range.

The 95%RI of all 12 HRT parameters with its associated 95% confidence intervals (95%CI) were calculated. Two methods were used to calculate 95%RI. If the distributions of the variables are normal and not significantly skewed we can calculate the mean and standard deviation (s) and the 95%RI is

$$95\%RI = mean \pm 1.96s$$

The standard error (se) and 95% confidence interval (95%CI) for the 95%RI limits can also be computed with the following formula.[56]

95%CI of the reference limit = reference limit ± 1.96se

where se = $\sqrt{s^2\{1/n + 2/(n-1)\}}$
for large n, se is approximately $\sqrt{3s^2/n}$

For variables that were not normally distributed or very skew, we can use the distribution-free (percentile technique) to directly estimate the 95%RI without any distributional assumptions. This percentile technique gives an unbiased estimate whatever the distribution and many investigators have advocated the use of this approach. To estimate the 95%RI we just calculate the $2.5^{th}$ and $97.5^{th}$ percentile.

### 3.10.5 Influence of Age on HRT Parameters

Regression analysis of age (as a continuous variable) to each of the HRT parameter was done to reveal any significant age-related influence on the measurements. The calculations were done by taking into account the sampling weights and stratification of each age group.

### 3.10.6 Statistical Softwares

Intercooled *Stata* 6.0 for Windows (Stata Corporation, College Station, TX, USA) was used for statistical analysis. Some graphical and exploratory data analysis were done using *NCSS* 2000 for Windows (NCSS, Kaysville, Utah, USA) and *SigmaPlot* 2000 for Windows 6.10 (SPSS Inc., Chicago, IL, USA).

## 3.11 Ethical Consideration

1) The subjects entered the study after a detail explanation of the process and an information sheet about the detail of the project was given (Appendix C). They can leave the study at any time. Informed consent had to be signed for every participated subject (Appendix D).

2) The study was approved by the Human Research Ethics Committee.

3) Subjects that were abnormal on examination were sent to the general eye clinic for proper management.

### 3.11.1 Potential Harm to the Subjects

All the procedures were routine examinations that were used at the eye clinic. The adverse effect was minimal. The potential adverse effects from pupil dilatation was reduced by avoiding to use 10% phenylephrine and use only 1% tropicamide.

### 3.11.2 Safety of the HRT

This is a very safe Class I laser with very low energy (25-50 microwatts) emission when scanning the retina. It has been approved by the U.S. FDA for use in human and has been used in many countries.