CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Question and Objective

3.1.1 Research Question

Does the modified hemoglobin H inclusion test has 90 percent sensitivity for screening alpha-thalassemia 1 trait among asymptomatic adults with positive one tube osmotic fragility test?

3.1.2 Research Objectives

3.1.2.1 To find the sensitivity of modifies the hemoglobin H inclusion test for screening asymptomatic people with α-thalassemia 1 trait.

3.1.2.2 To find the prevalence of α-thalassemia 1 trait in the first region of the Royal Thai Army.

3.1.2.3 To compare the cost-effectiveness ratio in term of cost per one case of α-thalassemia 1 detected

between modified hemoglobin H inclusion test and PCR test.

3.1.3 Operative Definitions:

α-thalassemia trait is found in a healthy person who can transmit alpha-thalassemia gene to his or her offspring. There are two types of α-thalassemia trait i.e., α-thalassemia 1 and α-thalassemia 2. Most of the α-thalassemia 1 is of a Southeast Asian subtype (--SEA/$\alpha\alpha$).

Prevalence of α-thalassemia trait for this study is defined as the case of α-thalassemia 1 trait found in the family of the first region of the Royal Thai Army.

Cost-effectiveness ratio is the comparison of the cost per one case of α-thalassemia 1 detected between modified hemoglobin H inclusion test and PCR test.

3.1.4 Key Words:

alpha-thalassemia, heterozygote, screening, cost-effectiveness.

3.2 Research Design

This is a diagnostic test. The performance of modified hemoglobin H inclusion test with positive osmotic fragility test will be done and compared with PCR test in asymptomatic people in the first region of the Royal Thai Army(appendix 1).

3.2.1 Design Overview

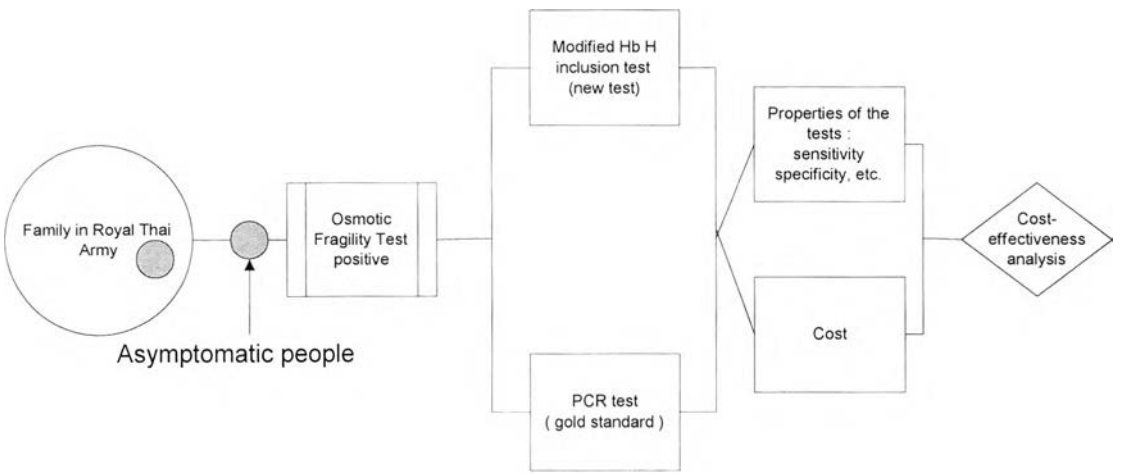

Figure 3.1. Design overview of screening test for α-thalassemia 1 trait in asymptomatic people with positive osmotic fragility test.

3.3 The Sample:

3.3.1 Target population

The target population of this study are asymptomatic people from the first region of the Royal Thai Army.

3.3.2 Sample population

The sample population are asymptomatic persons in the Medical Department, the first and eleventh infantry division from the first region of the Royal Thai Army who meet the following eligible criteria. The modified Hb H inclusion test will be done after positive result of one tube osmotic fragility test. All samples will be confirmed for α-thalassemia 1 trait of Southeast Asian type by PCR test and each result will be blindly observed.

3.3.3 Inclusion criteria

1. Asymptomatic people
2. Both sex and any age

3. Positive osmotic fragility test

4. Agreement to participate.

3.3.4 Exclusion criteria

1. Patient with liver disease who suffer from obstructive jaundice because interpretation of one tube osmotic fragility test is influenced by serum bilirubin.

2. Thalassemia disease, i.e. homozygous β-thalassemia, and β-thalassemia / Hb E

3.3.5 Sample size calculation :

$$N = \frac{Z\alpha^2 PQ}{\Delta^2}$$

N = Number of cases with α-thalassemia-1 trait.

$Z\alpha$ = Z-value at the level of α error (0.05) = 1.96

P = Probability of expected sensitivity of the screening test = 0.90

Q = 1-P = 0.10

Δ = Probability of error of sensitivity that can be accepted = 0.05

$$N = \frac{1.96^2 (0.90)(0.1)}{(0.05)^2} = 138.3$$

Since the prevalence of α-thalassemia trait is 30%, the sample size of the osmotic fragility test (OFT) should be :

$$= \frac{100 \times 138}{30} = 460$$

The previous pilot study[36] showed that 20% of the population were positive of osmotic fragility test (OFT). Therefore the sample size of this study should be :

$$= (460 \times 100) / 20 = 2,300$$

3.4 Data Collections :

This study started with a campaign of thalassemia prevention and control program. Firstly, the information of thalassemia screening was introduced to the people in the first region of the Royal Thai Army. Explanation was given to all participants after written informed consent was done(Appendix II). The person who would like to be screened were asked to fill the demographic data record form (Appendix III). A 10-milliliter blood sampling was drawn from the median cubital vein with aseptic technique. Modified Hb H inclusion test by method of

Jones JA,et al[28] was done after positive result of one tube osmotic fragility test.

3.5 Measurement

3.5.1 Demographic variables:

- name, address and identification number
- age, sex

3.5.2 Outcome variables

3.5.2.1 For one tube osmotic fragility test

Results

Positive is defined as a cloudy or smoky appearance.

Suspicious is considered to be a very fine cloudiness.

Negative is characterized by a clear red hemoglobin solution.

Interpretation:

Positive and Suspicious will include α-thalassemia1 trait, β-thalassemia trait, and iron deficiency anemia

Negative = normal

3.5.2.2 For modified hemoglobin H inclusion test

Results are reported as the number of red cells that contained hemoglobin H inclusion bodies which were found by scanning under oil immersion for 20 minutes at x100 magnification.

Positive is defined as one or more red cell containing hemoglobin H inclusion bodies.

Negative is defined as having no red cell containing hemoglobin H inclusion bodies

Interpretation:

Positive will include α-thalassemia 1 trait, α-thalassemia 2 homozygous and Hemoglobin H disease

Negative = normal

3.5.3 Gold standard

All blood samples, which were positive from the OFT, were confirmed by PCR test[11] for diagnosis α-thalassemia 1 trait of Southeast Asian type. For the other causes of microcytic anemia which can give the results of osmotic fragility test positive such as iron deficiency and beta-thalassemia, hematologic measurement were performed on an electronic cell counter (Coulter model STKS). Hemoglobin A2 were quantitated after elution from cellulose-acetate electrophoresis[37]. Hemoglobin type will be performed on starch gel electrophoresis and serum ferritin determined by ELISA test[38].

3.5.4 Data Analysis

3.5.4.1 Summary of demographic variables:

These variables were reported in a percentage, proportion, mean and standard deviation.

3.5.4.2.Calculation of outcome variables:

The dichotomous data of both the modified hemoglobin H inclusion test and the PCR test were analyzed by two by two tables in order to determine their performance. These variables were reported in nominal scale(positive and negative).

Table 3.1. The two by two tables of the modified hemoglobin H inclusion test and the PCR test.

		PCR test for α-thalassemia 1	
		Present	Absent
Modified Hb H inclusion test	Positive	a	b
	Negative	c	d

The sensitivity is defined as the proportion of people whose test of α-thalassemia trait are positive. It is calculated by the following formula :

$$\text{Sensitivity} = a / (a + c)$$

The specificity is defined as the proportion of people who are negatively tested for the α-thalassemia trait. It is calculated by the following formula :

$$\text{Specificity} = d / (b + d)$$

Positive predictive value (PPV) is the probability of the α-thalassemia trait in the people with a positive

(abnormal) test result. It is calculated by the following formula :

$$PPV = a / (a + b)$$

Negative predictive value (NPV) is the probability of not having the α-thalassemia trait in the people with negative (normal) test result. It is calculated by the following formula :

$$NPV = d / (c + d)$$

Accuracy is the proportion of all test results, both true positive and negative, that are corrected. It is calculated by this formula :

$$Accuracy = (a + d) / (a + b + c + d)$$

Another alternative to describe the performance of diagnosis test is the likelihood ratio. The likelihood ratio is defined as the probability of the test result in the present of α-thalassemia trait divided by the probability of the result in people without α-thalassemia trait. Likelihood ratios express how many times more (or less) likely a test result is to be found in α-

thalassemia, as compared to normal people. It can be calculated by the formula below :

$$\text{Likelihood ratio for positive test (LR+)} = \frac{a \,/\, (a + c)}{b \,/\, (b + d)}$$

$$\text{Likelihood ratio for negative test (LR-)} = \frac{c \,/\, (a + c)}{d \,/\, (b + d)}$$

$$\text{Prevalence} = a + c \,/\, (a + b + c + d)$$

3.5.4.3 Economic Evaluation

Description of alternative

There are two types of test for detection α-thal 1 trait among osmotic fragility test positive screenee that is modified hemoglobin H inclusion test and PCR test.

Alternative 1: Modified hemoglobin H inclusion test. The positive results must be confirmed by the PCR test.

Alternative 2: PCR test

Because this is a gold standard, the positive and negative results are α-thal 1 trait and normal respectively.

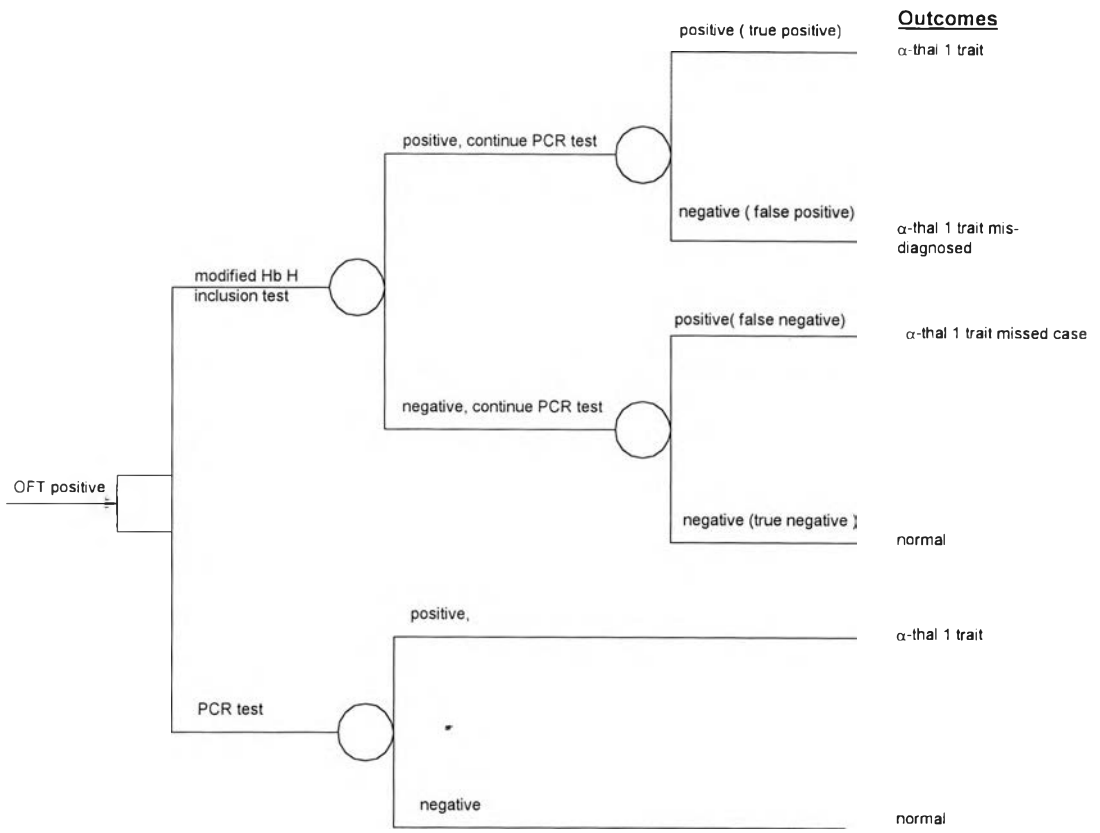

Figure 3.2. A decision tree for α-thalassemia-1 trait detection.

View point for the study

The view point of this study is provider perspective (government).

Cost Analysis

Cost were determined for the fiscal year 1998 and expressed in Baht for each intervention encountered in each alternative. Two different types of cost were estimated fixed cost and variable cost.

1. Fixed cost

The fixed cost or capital depreciation cost (CC) consists of building, machine and special machine cost.

2. Variable cost

Direct cost

The direct cost consists of labour cost (LC) and material cost (MC).

Indirect cost

In view point of provider, there is no indirect cost.

Total cost calculation = LC + MC + CC

3.5.4.4 Cost effectiveness ratio

Cost effectiveness ratio = $\frac{\text{(total cost of each program)}}{\text{(case detected by each program)}}$

Incremental cost=(Cost $_{PCR}$ - Cost $_{Hb\ H}$)/(Case $_{PCR}$ - Case $_{Hb\ H}$)

Cost $_{PCR}$ = total cost detected by PCR test

Cost $_{Hb\ H}$ = total cost detected by modified Hb H test

Case $_{PCR}$ = case detected by PCR test

Case $_{Hb\ H}$ = case detected by modified Hb H test

3.6 Ethical Considerations :

3.6.1. Explanation were given to all participants of this study.

3.6.2. Drawing 10 ml. of blood from the subject of the study did not cause any health problem in adult people.

3.6.3. All of the test results were kept secretly and were informed and clearly explained to those people who participated individually.

3.7 Limitations:

3.7.1. This technique took time about 3 hours per case to find red cell containing inclusion bodies.

3.7.2. This modified preparation which used enriched young red cells obtained both red cell containing inclusion bodies and reticulocytes which will influence the interpretation. By careful examination, hemoglobin H inclusion bodies appeared as multiple, bluish-green, dotted positively of "golf" pattern, whereas the reticulocyte showed bluish-purple granule or reticular network.

3.8 Expected Benefit and Application :

3.8.1. If the modified hemoglobin H inclusion test has approximately 90% sensitivity or more to detect α-thalassemia 1 of the Southeast Asian type, this test can be applied in those clinical laboratories which have very limited facilities.

3.8.2. Both the equipment and reagent needed in this test are available in most laboratories and not expensive. It will be very useful in the field studies.

3.8.3. This test can be combined with one tube osmotic fragility in order to screen α-thalassemia 1 trait effectively.

3.8.4. This test is the most important screening part for national thalassemia prevention program, which can prevent new case of thalassemia effectively.

3.9 Administration and Time Schedule:

It took 4 months for data collection, two months for data analysis, two months for writing thesis and another month for correction and preparation for final presentation.

Table 3.2. The Administration and Time Schedule

year/month / activity	19 98											
	1	2	3	4	5	6	7	8	9	10	11	12
pre-proposal												
standardization of test												
training personnel												
data collection												
data analysis												
thesis writing												
correction and final presentation												