# CHAPTER 2

# LITERATURE REVIEW

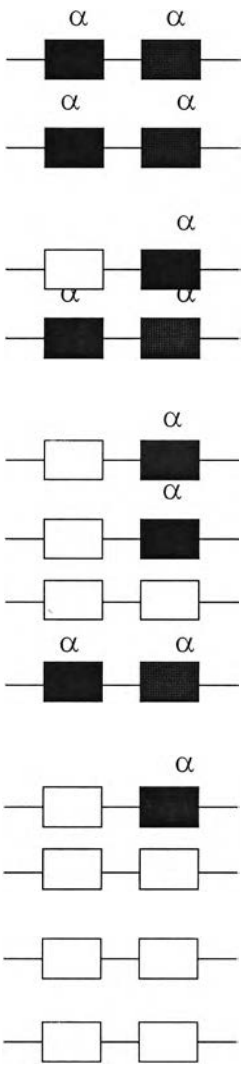
## 2.1 Introduction

Thalassemia are the commonest single gene disorders[12]. They are heterogeneous group of hemoglobin synthesis, characterized by the absence or reduce output of one or more of the globin chain of hemoglobin. They can be classified according to defective globin chain synthesis into $\alpha,\beta,\delta,\delta\beta$ and $\varepsilon\delta\gamma\beta$ thalassemia. Clinical manifestations are diversities, ranging from asymptomatic to profound anemia, which is fatal in utero or neonatal death.

In Southeast Asia especially in Thailand, there are high incidence of thalassemia and hemoglobinopathies [13]. The frequency of $\beta$-thalassemia is 3-9% and Hb E incidence is remarkably reaching 53% in certain part of the country. The frequency of $\alpha$-thalassemia reaches 20-

30%. It has been estimated that about 1% of the Thai population are affected with thalassemia disease[14,15].

The $\alpha$-thalassemia is defined as a group of genetic disorders which result in defective $\alpha$-chain synthesis[5].Because there are two $\alpha$ globin genes per haploid chromosome, the $\alpha$-thalassemia are classified according to the relative output of both $\alpha$ genes. Where both $\alpha$ globin genes on a chromosome are inactivated, the condition is call $\alpha^0$ thalassemia or $\alpha$-thalassemia 1 ($\alpha^0$ thal or $\alpha$-thal 1).One of these, the Southeast Asian type of deletion of both $\alpha$ globin gene ( - - SEA ) is the most common form detected in Thailand[16]. The heterozygous genotype can be written -- SEA / $\alpha\alpha$. When one of the linked $\alpha$ globin genes is inactivated the condition is called $\alpha^+$-thalassemia or $\alpha$-thalassemia 2 ($\alpha^+$ thal or $\alpha$-thal 2), and genotype can be written -$\alpha$ / $\alpha\alpha$. The genotype of $\alpha$-thalassemia is shown in figure 2.1.



| Condition / Disease | Genotypes |
|---|---|
| Normal | αα / αα |
| Heterozygous α-thalassemia 2 | -α / αα |
| Homozygous α-thalassemia 2 | -α / -α |
| Heterozygous α-thalassemia 1 | -- / αα |
| Hemoglobin H disease | -α / - - |
| Hb Bart's hydrops fetalis | - - / - - |

**Figure 2.1** Model of α-globin gene deletion as molecular basis for the various α-thalassemia syndromes. Gene deletions are represented by the white blocks.

Clinically, there are two important types of α thalassemia. First, there is the *Hb Bart's hydrops fetalis* which results from the homozygous state for α-thal 1. The majority of the mother bearing such hydrops

have toxemia of pregnancy and the cause of death of hydrops fetus is due to physiologic dysfunction of Hb Bart's. Second, there is a condition of varying severity called *Hb H disease* which is the compound heterozygous for $\alpha$-thal 1 and $\alpha$-thal 2. The clinical features are anemia, jaundice, and hepatosplenomegly but the most prominent manifestation is acute hemolysis after infection.

At present, in dealing with Hb Bart's hydrops fetalis, prevention is the most important. In setting up a population screening program it is obviously important to consider the relative cost and accuracy of the available methods. Screening for $\alpha$-thalassemia 1 can be performed by many methods.

Family study is an easy way if there had been a previous affected child with hemoglobin Bart's hydrops fetalis. Since this condition is transmitted as autosomal recessive, their parents are obligatory $\alpha$-thal 1 trait. This method was limited to family with index cases. It was not used to screen in general population.

Hemoglobin electrophoresis has been used to detect small amount of hemoglobin Bart's in newborn babies. Hemoglobin Bart's detection in cord blood[5,17-19] is relatively an easy way of screening for α-thalassemia 1, but it can not be applied in general population because Hb Bart's can not be found in adult with α-thalassemia 1 carrier.

Immunological method has been developed for the same purpose. Wasi P,et al [20] ,used anti Hb Bart's antibody produced in a rabbit after weekly injection with hemolysate from Hb Bart's hydrops fetus. This technique could detect α-thalassemia carrier, but it is not practical because it can not differentiate α-thal 2 from α-thal 1 trait. Since the embryonic ζ-globin chain is raised in red cells of Southeast Asian adult who has - - SEA deletion, this is detectable by a highly specific anti-ζ monoclonal antibody reaction. By using these techniques, minute a mount of ζ-globin chain can be detected in individual adults who are carriers of α-thalassemia 1 due to the - - SEA deletion[21-23].This technique is more specific but expensive.

The definite diagnosis of $\alpha$-thalassemia 1 is restriction endonuclease mapping by Southern blot technique[4], but it is laborious, radioactive and expensive.

The application of a polymerase chain reaction (PCR) base protocol[24,25] which specially detects the $\alpha$-thalassemia 1 of Southeast Asean type (- -SEA deletion) may soon supersede that of gene mapping. The possibility of applying this PCR base method[9] in routine screening for $\alpha$-thalassemia 1 may be alternative method because it is rapid and nonradioactive. The limitation of this test is expensive and highly technological.

Inclusion of cell containing hemoglobin(Hb) H with supravital redox dyes results in oxidation and precipitation of the soluble Hb H as inclusion bodies within the cell. This conventional Hb H inclusion test[26] is another method for diagnosis of $\alpha$-thalassemia 1 carrier, using one part of 1% brilliant cresyl blue mixed with two parts of blood and incubated at $37^0$ C for 1-2 hours. Dried blood smear is then examined under oil immersion, but Maungsapaya W, et al[27] and Chan AYY, et al

[9] reported the sensitivity of Hb H inclusion test were 50% and 79% respectively. An improved method for detection of red cell hemoglobin H inclusions described by Jones JA, et al[25] can detect α-thalassemia carrier approximately twice as compared with conventional method.

Skogerboe KJ,et al[29] studied the correlation of hemoglobin H inclusion bodies with DNA-determined genotype. They demonstrated that α-thalassemia 1 trait had inclusion bodies positive more than 50 cells per 50,000 enriched red blood cells by the modified technique ( table 2.1 ).

**Table 2.1**.Comparison of genotype, clinical, Hb H inclusion and erythrocyte indexes in α-thalassemia syndromes.* per 50,000 enriched red blood cells.

| Genotypes | Clinical expression | Hb H* inclusion | MCV |
|---|---|---|---|
| αα / αα | Normal | 0.1(0-1) | 84.6 |
| -α / αα | α-thal 2 trait, asymptomatic | 0.8(0-3) | 76.8 |
| -α / -α | homozygous α-thal 2, asymptomatic | 1.3(0-6) | 68.5 |
| --/ αα | α-thal 1 trait, asymptomatic | >50(20-173) | 68.3 |
| --/ - α | Hb H disease, anemia, splenomegaly | >90% | 61.0 |

There are two steps or serial tests for screening alpha thalassemia. The most commonly test used for the first screening test is MCV or osmotic fragility test. The results include three conditions, i.e. iron deficiency anemia, alpha and beta thalassemia carrier. Kattamis C, et al[30] demonstrated that osmotic fragility test could detect iron deficiency, beta and alpha thalasssemia with sensitivity 80, 98, and 80% respectively. Because the properties of screening test should be simple, fast, reliable and low cost[31], the second test for detecting alpha thalassemia is hemoglobin inclusion test. Modification of this screening technique produce a high sensitivity. The sensitivity of this modified method[32] was 91% for detecting obligatory $\alpha$-thalassemia trait without false positive results. Lin CK, et al[33] used the same technique for study prevalence of $\alpha$-thalassemia trait in the Taiwanese population. A total of 1435 healthy people were randomly screened by the mean corpuscular volume (MCV). The subjects with MCV less than 80 femtolitre (fl) were then performed by modified hemoglobin H inclusion test. The sensitivity and specificity for detecting $\alpha$-thalassemia 1 were 100 and 82% respectively.

The objective of screening test is to detect carriers who are at risk of having an affected child if the other parent is also a carrier. Ideally, such screening should be done before pregnancy in order to allow a wide choice of reproductive option, including avoidance of marriage to another carrier, having no child, or the use of a sperm donor, in addition to antenatal diagnosis with selective abortion of affected fetuses[34,35]. In Thailand there are no studies using these serial screening test. Because both the osmotic fragility and modified hemoglobin H inclusion test are simple, fast, low cost and high sensitivity, it should be appropriate for screening $\alpha$-thalassemia trait.