CHAPTER II

REVIEW OF RELATED LITERATURE

The inherited disorders of hemoglobin, the hemoglobinopathies, are genetic disorders of structure and synthesis of one or more of the globin polypeptide chain. Hemoglobinopathies can be divided into a number of overlapping groups: (1) the structural hemoglobin variants that involve substitution, addition, or deletion of one or more amino acids of the globin chain; (2) the thalassemias, a group of disorders in which there is a quantitative defect in globin gene production; (3) combinations of (1) and (2) that result in complex hemoglobinopathies; and (4) hereditary persistence of fetal hemoglobin (HPFH), an asymptomatic disorder (Kaplan and Pesce, 1989).

DEFINITION AND CLINICAL MANIFESTATION OF THALASSEMIA

Thalassemias are inherited hemoglobinopathies resulting from a decreased rate of production of one or more of the globin chains of hemoglobin. They are quantitative hemoglobinopathies that differ from the qualitative hemoglobinopathies by the fact that the structure of the affected globin chains (or chains) is normal but its synthesis results in decreased red cell hemoglobin, hypochromia, and a

variable hemolytic component (Kaplan and Pesce, 1989).

The clinical syndromes associated with thalassemia arise from combined consequences of inadequate hemoglobin accumulation and unbalanced accumulation of globin subunits. The former causes hypochromia and microcytosis, the latter leads to ineffective erythropoiesis and hemolytic anemia. Clinical manifestations are diverse, ranging from asymptomatic hypochromia and microcytosis to profound anemia, which is fatal in utero or in early childhood if untreated (Schwartz and Benz., 1991).

EPIDEMIOLOGY

Thalassemia have been encountered in virtually every ethnic group and geographic location. However, they are most common in the Mediterranean basin and equatorial or near equatorial regions of Asia and Africa. The "thalassemia belt" extends along the shore of the Mediterranean and throughout the Arabian peninsula, Turkey, Iran, India and southeastern Asia, especially Thailand, Cambodia, and southern China. Gene frequencies in these regions range from 2.5 to 15 percent (Schwartz and Benz., 1991).

In 1983 the population in south-east Asia was approximately 338 million and the numbers of people with α thalassemia and β thalassemia trait were estimated respective-

ly as 16,121,000 and 11,445,000. In Indonesia the prevalence of α thalassemia trait, ß thalassemia trait and Hb E trait (hemoglobinopathies) were estimated respectively to be 0.5; 3 and 4 percent (Boon, 1983). Studies in some areas in Indonesia show that prevalence of ß thalassemia trait is 3 - 7.8 percent (Sofro *et al.*, 1986; Pramudji Hastuti *et al.*,1991; Wahidiyat I., 1992).

PROBLEM OF THALASSEMIA IN INDONESIA

Both ß thalassemia major and double heterozygous ß thalassemia/Hb E can give rise to a serious social problem because the cost for their treatment is very high. An example of ideal treatment would include blood transfusion once in 2 - 3 weeks (Hunstman, 1987; Kattamis, 1985) and subcutaneous infusion of deferoxamine hydrochloride in 1978 the cost is up to US $ 5,000 per-child, per-year in Greek and US $ 7,513 in United Kingdom (WHO working group, 1982).

Based on a prevalence of ß thalassemia trait 3 - 5 percent, total population 150 million and the birth rate 23 per 1000, Wahidiyat (1992) estimated that not less than 2000 newborn would suffer from thalassemic syndrome in Indonesia each year. Then cost of treatment (if given) for those patients per-year can also be estimated at US $ 10 million.

Without any prevention program, Indonesia may also face a problem with blood donors similar to that which had occurred in Cyprus. In Cyprus, with a total population 600,000 and the prevalence of ß thalassemia trait was 17 %, 1 in 35 marriages was at risk, and theoretical birth rate of homozygoses was 1 in 138. As modern treatment was available they made forward projection of the cost of treatment and the need of blood donation. They predicted that within 20 years, more than 40 % of all possible blood donors might have to give blood annually for the treatment of thalassemia alone. (WHO, 1983).

METHODS FOR SCREENING FOR ß THALASSEMIA TRAIT

The most commonly tests used for screening for ß thalassemia are MCV and O.F.T. (Schwartz and Benz., 1991; Boon W.B., 1983). For this purpose some center use MCV (Pearson H.A. *et al.*, 1973; Maccioni and Cao, 1985; Cao A. *et al.*, 1984; Shine and Lal, 1977) and some other centers use O.F.T. (Silvestroni and Bianco, 1983; Anichini *et al.*, 1983; Flatz and Flatz, 1980). Another uncommon test for screening is done by measuring volumetric distribution of blood corpuscles using an electronic equipment. This method is able to distinguish subjects with ß thalassemia trait from normal and sideropenic subjects (Torlontano *et al.*, 1972). Other values indicate thalassemia trait are (1) (0.23 X MCV) - (0.22 X RBC) - (0.93

X hemoglobin) - 3 less than 0; (2) MCV:RBC ratio less than 13 and (3) MCV^2 X MCH less than 15 (Brewer G.J. *et al.*, 1993).

OSMOTIC FRAGILITY TEST (O.F.T.)

O.F.T. runs by using 12 concentrations of saline solution can be used to diagnose hereditary spherocytosis, ß thalassemia major, ß thalassemia minor, hemoglobin E disease and other thalassemias (Sonneenwirth and Jarett, 1980). In those people who suffer from thalassemia and hemoglobin E (Hb E) disease, the resistance of erythrocytes to hypotonic saline solution are increased, whereas in hereditary spherocytosis its resistance is decreased. This is influenced by the surface and volume ratio of erythrocytes. If this ratio is increased the resistance will increase (Maslow *et al.*,1980; Henry, 1991; Dacie and Lewis, 1984).

O.F.T. had been modified several times to make it easier to run, read and interpret this test. For an example, Danon in 1963, read the result test automatically using a continuous decrease of salt concentration. In order to detect thalassemic patients Malamos *et al.*, 1962 used one tube test containing 5 ml of 0.4 % saline and 20 ul of blood and then read visually. But other studies used 0.36 % (Flatz and Flatz, 1980) and 0.35 % of saline solution (Valbonesi *et al.*, 1980). Flatz and Flatz, in 1980 used the time to 50 %

hemolysis in a glycerine-saline solution. This method had a sensitivity of 100 percent and a specificity of 79.1 %. Then, Valbonesi *et al.* in 1983, used nephelometric test for quantification of osmotic fragility of erythrocyte.

The recommended sample for the O.F.T. test is defibrinated or heparinized blood. A blood sample with oxalate or citrate anticoagulant should not be used because it will cause the concentration of salt in the blood becomes higher, if used. The blood samples should be tested within two hours but this can be postponed up to six hours if the blood sample is kept in 4^0 C. This test should be done in room temperature (Dacie and Lewis, 1984).

CHARACTERISTIC TEST FOR SCREENING

There are many criteria for successful screening program : it should be simple, fast, reliable, (Silvestroni and Bianco, 1983) specific yet not expensive or time consuming (Flatz and Flatz, 1980). The objective of the use of diagnostic tests for screening is to detect disease in its earliest, presymptomatic state when, presumably, it is less widespread and more easily treated or cured.

The test for screening would share many of characteristics of ideal diagnostic test in general. Sensitivity, specificity, and predictive values should all

close to 100 percent. But such tests do not exist. Therefore, sometimes test with high sensitivity or high specificity have to be chosen. Each choice has a different consequence.

The test with high sensitivity has few false negatives and has a high negative predictive value. The tradeoff, unfortunately, is that this type of test has poor positive predictive value, that means most positive individuals would be falsely positive. When such a test is used for screening for large populations, a significant number of individuals have positive test but only a small number of them are truly diseased. The consequence of choosing this test is that resulted in a high proportion of unnecessary diagnostic evaluations. The test with high specificity will have higher positive predictive values, but lower negative predictive values. The consequence of choosing this test is that resulted in sizeable proportion of diseased individuals going undetected. The choice will depend on whether or not patients will benefit from early disease detection, as well as the relative cost and risk of subsequent diagnostic evaluation (Kaplan G., 1990).

ß THALASSEMIA TRAIT

The heterozygous state for ß thalassemia is not usually associated with any clinical disability except in periods of stress, such as pregnancy or during severe infection, when a moderate degree of anemia may be present (Weatherall D.J., 1983).

The diagnosis is indicated by the hematologic picture of mild hypochromic microcytic anemia in the presence of adequate iron stores. Hemoglobin values are usually in the 9 to 11 g/dL range (Weatherall D.J., 1983) or 1 or 2 g/dL lower than that seen in normal persons of the same age and sex (Schwartz and ., 1991). The most striking and consistent finding is small, poorly hemoglobinized red cells i.e. MCH values of 20 to 22 pg (Weatherall D.J., 1983); less than 26 pg (Schwartz and Benz., 1991); less than 27 pg in males and less than 26 pg in females (Cao A. *et al.*,1984) and MCV values of 50 to 70 fl (Weatherall D.J., 1983); less than 75 fl (Schwartz and Benz., 1991); less than 79 fl in males and less than 77 fl in females (Cao A. *et al.*, 1984); less than 79 fl (Pearson H.A. *et al.*, 1973). MCV values greater than 75 fl can be used to rule out thalassemia (Kaplan L.A. and Pesce A.J. 1989).

A major help in the diagnosis is elevated levels of Hb A_2. This minor hemoglobin usually less than 3.5 percent of

total hemoglobin but in ß thalassemia is usually 5 percent or higher (Brewer G.J. *et al.*, 1993); more than 3.5 percent (Pearson H.A. *et al.*, 1973); more than 4 percent (Cao A. *et al.*, 1984). The average level of Hb A_2 is 5.1 percent and range 3.5 to 7.0 percent (Schwartz and Benz., 1991; Weatherall D.J., 1983). This value may be artificially depressed in patients with ß thalassemia who have coexistent iron deficiency, and it may rise into the ß thalassemia with the institution of iron therapy.

Hemoglobin F (Hb F) levels are inconsistently elevated in this disease. In about half of the cases Hb F is in the normal range (less than 2.0 percent); in the remainder they are moderately elevated around 2.1 to 5.0 percent (Schwartz and Benz., 1991).

Red cells count is increased or normal and in the smear shows varying numbers of target cells, poikilocytes, ovalocytes and basophilic stippling (Schwartz and Benz., 1991).

α THALASSEMIA

In contrast to ß thalassemia, α thalassemia is due to a deletion mutation. There are four types of α thalassemia i.e. hydrops fetalis (α thalassemia-1 homozygosity); Hemoglobin H disease; α thalassemia trait (α thalassemia-1 or

α thalassemia^{0}) and silent carrier (α thalassemia-2 or α thalassemia^{+}). This study is more concerned with α thalassemia trait than the silent carrier state.

1. α thalassemia trait

The clinical and hematologic picture is very mild. Bart's hemoglobin is present, in the newborn period no hemoglobin H is demonstrable by electrophoresis, although hemoglobin H inclusion can be seen in occasional red cell. Hb H and Bart's hemoglobin are present in increased amounts, but 50 percent or more of hemoglobin will be A. If the syndrome is caused by an abnormal hemoglobin such as Constant Spring, it will be present but in low amounts due to the fact that the mutant will be of a type to reduce synthesis of the hemoglobin (Brewer G.J. *et al.*, 1993).

2. Silent carrier

Red cell morphology is normal and there are no clinical manifestations. Usually some Bart's hemoglobin will be present. If the allele is an abnormal thalassemic hemoglobin such as Constant Spring, that hemoglobin will be present but in low amount due to the fact that the mutant will be of a type to reduce synthesis of hemoglobin (Brewer G.J. *et al.*, 1993). Schwartz and Benz., 1991 reported that there is no simple screening procedure that will detect this disease

but Cao A. *et al.*, 1984 claimed to and miss only a few silent carrier cases by using their own screening procedure.

α thalassemia, which occurs in the same population as β thalassemia makes screening more complicated. This diagnosis suggested by a mild, familial hypochromic microcytosis with low or normal levels of Hb A_2 and Hb F and no incidence of iron deficiency. Precise diagnosis requires demonstration of α globin gene deletion or high β/α globin synthesis ratio (Schwartz and Benz., 1991).

HEMOGLOBIN E DISEASE ($\alpha_2\beta_2^{26Glu\text{-->}Lys}$)

Hemoglobin E (Hb E) is a common variant occurring in 15 to 30 percent of the population in Cambodia, Thailand, part of China and Vietnam (Schwartz and Benz., 1991). Hb E trait is asymptomatic, with microcytosis (average MCV = 73 fl) resembles very mild β thalassemia trait. Homozygoses are still asymptomatic and exhibit more microcytosis (average MCV = 67 fl) and erythrocytosis and slight,if any, anemia (Schwart and ., 1991 ; Henry J.B.,1991). Target cells are numerous on the blood film (Henry J.B.,1991). Compound heterozygotes for Hb E and a β thalassemia (β thalassemia/Hb E) resemble patients with β thalassemia intermedia or β thalassemia major (Weatherall D.J., 1983; Schwartz and Benz., 1991; Henry J.B., 1991). In this disease Hb A is reduced or absent (Henry

J.B.,1991).

Hb E is very mildly unstable, but this instability does not alter red cell life span significantly. The high frequency of the Hb E gene is due to the thalassemia phenotype associated with its inheritance. It can be demonstrated to be unstable; it precipitates abnormally in the heat denaturation test and with isopropanolol (Schwartz and Benz., 1991). On alkaline electrophoresis, it migrates and takes place tightly to Hb A_2 fraction so that it is difficult to separate in order to quantify each of them (WHO working group, 1982). In quantification, both are treated together and classified as Hb A_2. Therefore, quantification of Hb A_2 from cellulose acetate membrane (CAM) electrophoresis can be used to diagnose these two diseases. Values of more than 15 percent and 75 percent are respectively used to diagnose Hb E trait and Hb E homozygoses.