CHAPTER I

INTRODUCTION

Thalassemia is a serious disease that can generally be diagnose in the first year of life. The most common signs of thalassemia are anemia, hepatospleenomegaly, growth retardation, jaundice, and bone changes. All thalassemia patients that show acute clinical symptoms [1] are treated with hypertransfused whereby they regularly receive blood to keep a minimum hemoglobin level of 9-10 g/dl. Treating thalassemia can lead to chronic iron overload in patients and can especially lead to the development of diabetes [2, 3, 4, 5] and this often also accompanied by endocrine complications, iron bind to interstitial cell of the pancreas which results in increased fibrosis [3, 5]. These affect the microvascular circulation that leads to β-cell hypoxia or anoxia, resulting in a state of insulin deficiency [4]

The structure of hemoglobin

Human hemoglobin is a heterotetramer protein, consist of two alpha and two beta subunits as shown in Fig 1. Each subunit contains a heme group, an iron containing compound that binds to oxygen.

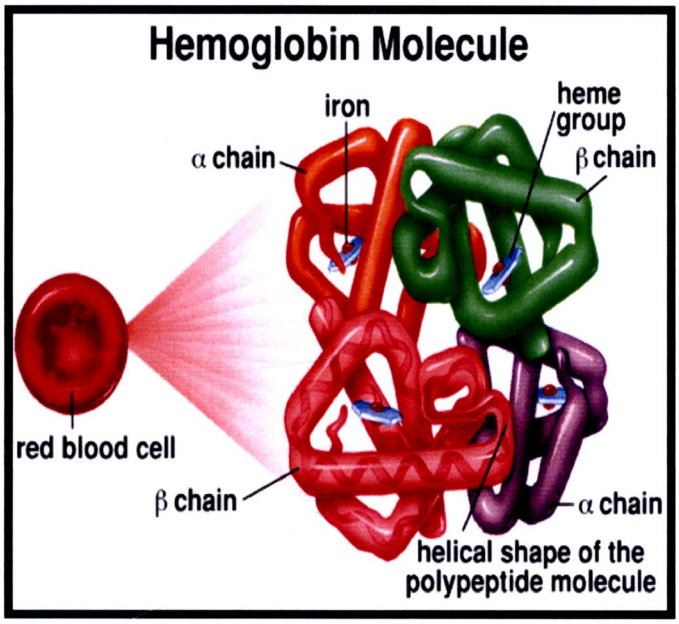

Figure 1 The Structure of Hemoglobin

Source: http://www.tikirobot.net/wp/tag/hemoglobin/

The synthesis of hemoglobin is controlled by two developmentally regulated multi-gene clusters: The alpha-like globins cluster on chromosome 16 and the beta-like globins cluster on chromosome 11 as demonstrated in Figure 2.

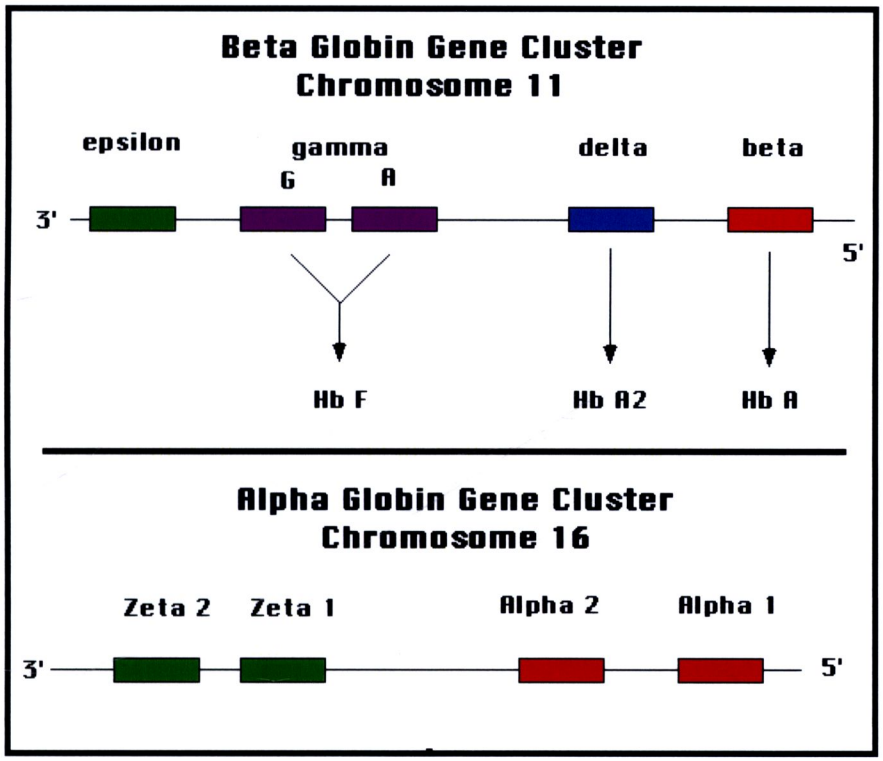

Figure 2 Beta globins gene cluster and Alpha globins gene cluster

Source: http://www.sickle.bwh.harvard.edu/hbsynthesis.html

In healthy persons, the synthesis of alpha and beta globins chain is finely balanced during terminal erythoid differentiation but the mechanism of balanced expression is unknown [6].

Genetic control of hemoglobin synthesis

There are six different globins chains (α, β, γ, δ, ε, ζ) are known to occur in normal human hemoglobin's at various stages of development.

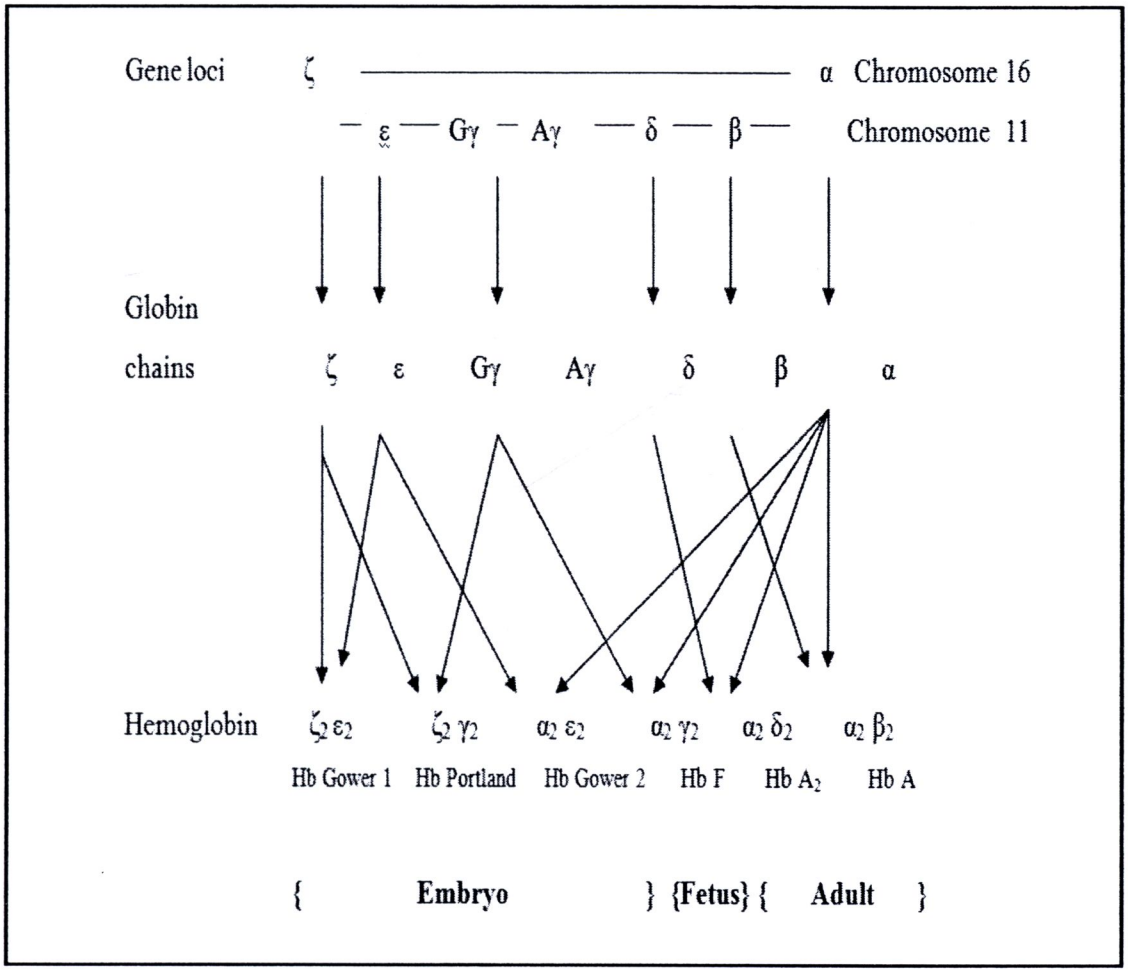

Figure 3 The genetic control of human hemoglobin [7]

Source: http://www.writer.dek-d.com

The α–like globins cluster is situated near the telomere region of the short arm of chromosome 16 and includes the ζ, α_2 and α_1 globin genes. The β – like globin cluster is interstitial and located on short arm of chromosome 11, with includes the ε, γ, δ and β globin genes [7].

Heme synthesis

The biosynthesis of heme in red-cell precursors takes place in a series of enzyme controlled steps, beginning with the condensation of glycine and succinyl-CoA to form α-amino β-keto adipic acid, which is then rapidly decarboxylated to form δ-aminolaevulinic acid (ALA). This reaction, which is catalyzed by enzyme ALA synthetase and requires pyrydoxal phosphate and ferrous ions as cofactors, is the only step in whole chain which requires energy, all the rest being essentially irreversible. Next, two molecules of ALA combine to form the monopyrrole porphobilinogen under the action of the enzyme ALA dehydrase, and then four of these porphobilinogen molecules are condensed to form the basic tetrapyrrole ring, uroporphyrinogen. After side chain modifications this is converted to protoporphyrinogen which, on oxidation by oxygen in presence of coproporphyrinogen oxidase, is finally converted to the red protoporphyrinogen molecule. Iron is then inserted by the enzyme heme synthetase to form heme [7].

The first and last two enzymes of this pathway are present in the mitochondria while the rest are found in the cell cytoplasm, and it is interesting to note that both oxidation steps take place in the mitochondria.

Normal Hemoglobins [8]

Normal human hemoglobin is expressed as A_2A. Hemoglobin A is the designation for the major hemoglobin protein that exists after birth that is a tetramer with two alpha globin chains and two beta globin chain ($\alpha_2\beta_2$). Hemoglobin A_2 is a minor component (less than 3.3%) of the hemoglobin found in red cells and consists of two alpha globin chains and two delta globin chain ($\alpha_2\delta_2$). The beta protein is not expressed before birth and the gamma hemoglobin (HbF) is the predominant hemoglobin protein found only during fetal development. Hemoglobin F is a tetramer molecule of two alpha chains and two gamma chains ($\alpha_2\gamma_2$). The genes for HbF and HbA are closely related and exist in the same gene cluster on chromosome 11.

Abnormal Hemoglobin

There are many types of abnormal hemoglobin (Table 1), with many results such as single-base substitutions, frame-shift variants, deletions and insertion, fusion genes, chain termination mutations and Nonsense mutations.

Table 1 Summary of molecular mechanism for structural hemoglobin variants

Mechanism	Example
Single-base change	Over 200 variants
Deletion of one or more residues	Hb Leiden ($\beta^{6 \text{ or } 7}$ Glu)
Frame-shift chain elongation	Hb Tak
Chain termination mutation	Hb constant spring
Fusion gene	Hb Lepore
Fusion gene	Hb Kenya

Many hemoglobin variants are of no clinical significance, however, a substitution at a critical part of the molecule which alters its physical properties and results in abnormal function or stability can give rise to a clinical disorder of varying severity. Such as homozygous α thalassemia, homozygous β thalassemia etc [7].

Thalassemia

Thalassemia is the one type of hemolytic anemia with a mutation of globins gene (reduced or absent amounts of hemoglobin). It was first recognized clinically in 1925 by Dr.Thomas Cooley, who described a syndrome of anemia with microcytic erythrocytes. Then it was called Cooley's anemia. Later Wipple and Bradford renamed this disease "Thalassemia". Because it was found in the region of the Mediterranean Sea (thalasa is an old Greek word for sea) [7].

Thalassemia can be separated into two major types.

1. α-thalassemia is caused by a change in the gene on the 16th Chromosome and results in the very low or non production of alpha globin chains.

2. β-thalassemia is caused by a change in the gene on the 11th chromosome and results in the very low or non production beta globin chains.

The prevalence of thalassemia has been reported in new cases at about the rate of 100,000 cases per year. Thalassemia is found in Italy, Greece, the Middle East, South Asia, Southeast Asia and Africa. β-thalassemia is found exclusively in the Mediterranean (including the south of Italy and Greece). Typically, 5-10% of the population is carriers and it is found in the populations of the Middle East, India, Pakistan and Southeast Asia. α-thalassemia is found exclusively in Thailand, China and the Philippines [7]. In Thailand the prevalence of thalassemia is about 1% of the population and 40% of the Thai population are carriers of this disease with asymptomatic [9].

The pathophysiology of thalassemia disease can range from asymptomatic to the fatal form. In severe α-thalassemia cases affected babies die within 6 hours of birth. Also in β-thalassemia, the symptoms can range from moderate to severe, depending in part on the exact genetic change underlying the disease. β-thalassemia major is diagnosed by clinical symptoms of severe anemia that can begin within months of birth. Severe anemia can result in severe lethargy, paleness and insufficient growth and development if left untreated. Other characteristic physical complications such as heart failure and enlargement of the liver and spleen can dramatically decrease life-expectancy [7].

Blood transfusion therapy, as needed, can be started as soon as anemia is detected. Transfusion was a regular treatment in thalassemia patients. For normal life-expectancy and to maintain the hemoglobin concentration do not go lower than 9–10 g/dl or hematocrit not lower than 27–30% to prevent face bone transformation [10]. This treatment requires blood transfusion every 4–6 weeks and must be giving together with iron–chelating therapy to prevent iron overload.

Because of continuous blood transfusions, thalassemia patients are subject to peroxidative tissue injury by secondary iron overload. Iron overload stimulates oxidative stress. As irons are catalyzing the Haber-Wiess reaction, the Fenton reaction changes the superoxide and hydrogen peroxide to be hydroxyl radical.

The goal of this research was to find the evidence and baseline of impaired glucose tolerance in transfusion-dependent β-thalassemia, and correlation with other variables of these patients. The measurement of impaired glucose tolerance will be useful for preventing of diabetes.

The hypothesis in this research is (i) Fasting blood glucose from oral glucose tolerance test in transfusion dependent β-thalassemia are higher than that of control. (ii) Fasting blood glucose from oral glucose tolerance test in transfusion dependent β-thalassemia after the 6 months interval is significantly higher than first state before.