CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

β – Thalassemia

β – Thalassemia or Cooley's anemia is caused by a change in gene for the beta globin component of hemoglobin. The symptoms can range from moderate to severs, depending in part on the exact genetic change underlying the desease. β-Thalassemia major is diagnosed on clinical symptoms of severe anemia that can begin within months after birth. The severe anemia can result in severe lethargy, and insufficient growth and development if left untreated. Other characteristic physical complications such as heart failure and enlargement of liver and spleen can dramatically decrease life-expectancy.

Globin synthesis in β thalassemia

The main pathophysiologic feature of β – thalassemia is the accumulation of unpaired α globin chains in erythrocyte pecurors and red blood cell. This accumulation alters cell membrane function and results in ineffective erythopoiesis and early celldestruction. The mutations are designated as β^0 where no β globin is produced while only small fraction of the normal amount of β globin is produced are called as β^+ mutation. More than 200 mutations causing β thalassemia have been described and, with the exception of a few deletions. In β – thalassemia, the synthesis of normal α globin chains from the unaffected α globin genes continues as normal, resulting in the accumulation within the erythoid precursors of excess unmatched α globin. The free α globin chains are not able to form viable tetramers and instead precipitate in red cell precursors in the bone marrow forming inclusion bodies. In Thailand, almost β^0 thalassemia caused by point mutation from base substitution and frameshift mutation. In north area of Thailand have found β^0 thalassemia (codons 41/42(-TCTT) frame shift mutation) as well as β^0 thalassemia (codons 17 (A-T) base substitution). The severity of β – thalassemia can range from silent to severe, depending on determination factors for example: Type of mutation, have defected with α globin gene.

B Thalassemia major

β Thalassemia major is the β thalassemia patients with have a severity of clinical phatophysiology. They are homozygous β thalassemia and some of β thalassemia/Hb E, with have severe anemia, Hb levels approximately 3-4 g/dl and have the clinical at age 1-2 years to be receive blood transfusion from child.

Iron metabolisms

Background

Approximately 4-5 g of iron can be found in the average adult man, most of which is contained in the hemoglobin molecule and other heme containing proteins (two-third). Most inorganic iron is in the ferric, Fe (III), state, the most stable oxidation states for iron. Dietary iron is absorbed preferentially from the proximal part of the small intestine (~1-2 mg/day) in the ferrous, Fe(II), state by divalent metal transporter-1 (DMT1) protein and is transferred to the circulation in which it is carried by transferrin. The excretion is regulated to be the same amount [11].

Iron is bound to transferrin in the circulation and transported around the body. Most of them are taken up by the developing erythrocytes in the bone marrow and incorporating into hemoglobin. Old or damaged erythrocytes are removed from the blood stream by the macrophages of the RE system and the iron is recycled back to plasma transferrin. Smaller quantities of iron are exchanged with the body's storage tissues, predominantly the hepatocytes of the liver. Other tissues also take up iron for their metabolic needs, for example, the synthesis of myoglobin in muscle. Dietary iron enters the body via the proximal small intestine in amounts equaling the iron lost from the body by the shedding of epithelial cells, particularly from the gastrointestinal tract, thus maintaining the body's iron homeostasis [12].

Transferrin

Some of the absorbed iron is retained in the gut mucosal cells within ferritin and other proteins and will be lost in the feces when these cells are shed. The rest enters the circulation and bound to transferrin. Transferrin also accepts iron released by the destruction of aged RBC, e.g. in the spleen (estimated about 20-25 mg/day). Circulating transferrin only accounts for about 3 mg of total body iron, but this iron

turn over about 10 times daily. Transferrin is a glycoprotein synthesized mostly in the liver. Transferrin has N-terminal and C-terminal domains, each of which tightly binds one atom of Fe(III) at pH 7.4. In healthy people, transferrin is usually no more than 20-30% loaded with iron. Hence human and other animal blood plasmas have considerable iron-binding capacity and their content of "free" iron is virtually zero. The affinity of transferrin for iron at pH 7.4 is slightly higher for the C-terminal domain than the N-terminal domain. The strength of binding decreases at lower pH. Transferrin can bind several metal ion other than Fe(III), including aluminum and bismuth with lower affinity [11]. A protein similar to transferrin, known as lactoferin, is found in saliva, vagianl mucous, seminal fluid, tears and other secretory fluids. Many similar iron binding proteins are known such as ovotransferrin in white egg, melanotransferrin in melanoma cells. Cells that require iron express transferring receptors on their surface: the more iron they want, the more receptors they make. Receptors bind iron carrying transferrin, which is internalized by receptor-mediated endocytosis and enters the cytoplasm in a vacuole. The contents of the vacuole are acidified by the action of proton pumping ATPase, which weaken iron binding to transferrin. The iron removed by iron-binding agents, which identity is uncertain. The iron-free transferring (apotransferrin) is then ejected from the cell to re-use. However, it seems that a small iron pool will be maintained as complexes with a variety of small molecules, such as nucleotides and citrate within the cytoplasm and subcellular organelles [13].

Ferritin [14]

Most intracellular iron is stored in ferritin. Mammalian ferritin consists of hallow protein shell, 12-13 mm (7-8 mm inside diameter), and composes of 24 subunits. The shell surrounds an iron core that can hold up to 4500 ions of iron per molecule, but usually has fewer. Trace of other metals can be present in ferritin, including copper [15]. Iron enters ferritin as Fe^{2+} which is oxidized to Fe^{3+} and deposited in the core as an insoluble hydrated ferric oxide. Iron can be released as Fe^{2+} *in vitro* by several agents including ascorbate, thiol, urate and reduced flavin. Iron enters and leaves through channels, which are two types: non-polar and polar. Ferritin

an be converted in lysosomes into an insoluble product called hemosiderin, robably by roteolytic attack [13].

Regulations of iron balance

There is a synchronized regulation of the synthesis of transferrin receptors and ferritin subunits in mammalian cells. This involves cytoplasmic iron-regulatory proteins (IRPs) which bind to iron-responsive elements (IREs) [16], special base sequences in the mRNAs of ferritin and of transferrin receptor. When intracellular iron levels are too low, IRPs bind to IREs. This binding stabilizes transferrin receptor mRNA so that more protein is made. By contrast, it prevents translation of ferritin mRNA. When there is enough cellular iron, mRNA for the transferrin receptor is rapidly degraded [17].

Hepcidin, an iron regulatory peptide, is a small antimicrobial peptide synthesized by hepatocytes and secreted into the circulation. It inhibits the efflux of iron from macrophages, intestinal enterocytes and other cell types. It also reduces iron absorption by causing the degradation of the enterocyte iron transporter ferroportin [18]. Hepcidin production is decreased in response to stimuli known to increase cellular iron release (e.g. iron deficiency, increased erythropoiesis) and increased under conditions where iron release is inhibited (e.g. iron loading, inflammation). Hepcidin level is decreased in thalassemia patients [19], the expression of hepcidin gene is suppressed by high levels of GDF15 (growth differentiation factor-15) [20]. Under most circumstances iron remains tightly bound to one of several proteins and many small molecules. Although the iron binding proteins effectively chelated iron and prevent any appreciable redox effects under normal physiological conditions, this protection can break down in disease states. The iron complexes with small molecules can promote hydroxyl radical formation *in vitro*. Transferrin releases its iron at an acidic pH, particularly in the presence of small molecular weight chelating agents, including ADP, ATP, and citrate [21]. Such condition are found in areas of active inflammation and during ischemia reperfusion injury and it is therefore likely that hydroxyl radicals contributes to tissue damage in these settings. Iron is released from ferritin by reducing agents including ascorbate and superoxide itself [21] and hydrogen peroxide can release iron from a range of heme proteins [22].

Iron overload and Iron toxicity of iron in vivo

The toxicity of iron is mediated, in part, by its catalysis of reactions which generate free hydroxyl radicals, propagators of oxygen-related damage [23]. Hydoxyl radicals induce lipid peroxidation of cellular organelles including mitochondria, lysosomes, and sarcoplasmic membranes. Evidence of peroxidant damage has been demonstrated in vivo in the tissues of iron-loaded animals and of thalassemic patients [24]. In very heavily iron-loaded patients transferin becomes fully saturated and a nontransferrin-bound fraction of iron becomes detectable in plasma [25]. Nontransferrin-bound iron may accelerate the formation of free hydroxyl radical and and facilitate uptake of iron by tissues [27]. There is an evidence of increase in low molecular weight iron in serum and in the intracellular transit pool of iron .This promotes peroxidative damage to cell and organelle membrane. In organ that accumulate excess iron, including the liver, pituitary gland, pancreases and partially susceptible to the iron-induced peroxidative damage that, ultimately, can lead to congestive heart failure, Which is the main course of death in thalassemia patient [28].

Diabetes

Diabetes is a condition in which blood glucose levels are high due to for example, lower than normal levels of insulin and this is termed diabetes mellitus type I. Another reason might be due to insulin resistance; in this case, insulin is unable to control high levels of glucose. Therefore, high levels of glucose are observed despite high levels of insulin production and this is termed diabetes mellitus type 2 (DM2).

Diabetes mellitus type I (DM1) can be found in both children and adults. In most adults it is due to a low level insulin production in the pancreas following certain viral infections. These infections may destroy the insulin producing cells and therefore cause a decrease in the insulin production.

Insulin's role is to cause the uptake of blood glucose into cells to be used as food. In the event of insulin shortage, certain organs will not function properly and this can lead to premature organ damage for example to the ocular nerve, the kidney and the vascular system. Having this type of diabetes will cause premature aging with a higher risk of premature death.

DM2 can afflict both children and adults. Predominantly these patients are also overweight or obese and they lack regular exercise. They may also a higher fatty content in their liver. Taken together these may cause insulin resistance. The pancreas will produce higher levels of insulin than normal to cause the uptake of glucose from the blood stream into the cells of various organs; however due to insulin resistance this is ineffective. Therefore, a shortage of cellular glucose occurs similarly to DM1; however the symptoms and disease complications occur much more slowly with DM2.

Insulin

Insulin is a hormone that controls glucose levels.

The pancreas produces pancreatic enzymes and the beta cells within the pancreas produce insulin. Normally, the pancreas produces 1 unit of insulin per hour except after meals, the pancreas will increase its production of insulin by more than 10 times normal. By snacking, eating large meals or extremely sweet, the pancreas must work harder in a day. This to eventually leads a decrease in beta cell in old age.

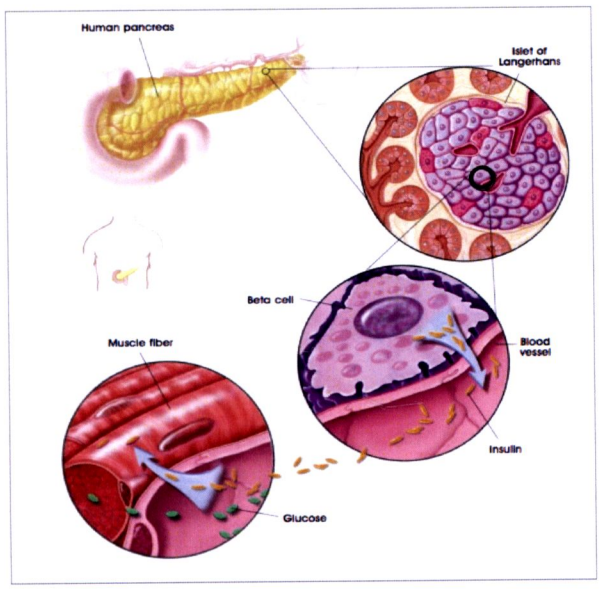

Figure 4 Insulin production by beta cells.

Source: http://www.stemcell.nih.gov

When the amount of glucose in the blood increases, beta cells will increase production of insulin. Insulin then binds onto its receptor on cell surfaces and glucose is taken up by these cells. If the number of receptors is diminished, for example when cells are overloaded with intracellular fat, a decrease in glucose uptake or an increase in leftover glucose in the blood stream is observed.

Insulin Sensitivity & Insulin Resistance

Rapidity at which, healthy cells react to insulin. Healthy cells react to increased insulin levels. However, when cells become less reactive to insulin or in other words insulin resistance, high levels of insulin in the bloodstream (hyperinsulinemia) are observed for a long time. In conditions where insulin receptors on cell surfaces are decreased for example in the model figure above where there are 20,000 insulin receptor sites and in the second figure, there are only 5000 receptor sites, there are sites for insulin to bind to since there are a limited amount of receptors. The amount of glucose that enters the cell is therefore less or in other words high levels of blood glucose are observed. When there are high levels of blood glucose, increased insulin production in triggered. Since insulin is an anabolic hormone (a hormone that causes positive metabolism), an increase in fat and protein synthesis occurs. When beta cells work hard for a long time, a decrease in beta cell function occurs and resulting in decreased insulin production. As such, glucose can no longer be cleared into the cells, which leads to a permanent diseased state of diabetes.

The diagnosis of diabetes can carried out by testing blood glucose content after fasting for at least 8 hours. If the levels of blood glucose are more than or equal to 126 mg/dl 2 out of 3 times, this is considered to be a clinical diagnosis of diabetes. Diagnosis of diabetes using these parameters is considered to be too late since with these blood glucose levels, approximately 50% of beta cell function will have been lost. If impaired glucose tolerance (IGT) is tested is when beta cells have lost less then 75% of their function and can be tested 10 years before the onset of diabetes. Results from the IDF (International Diabetic Federation) have found that 30% of the subjects in IGT group were able to have their blood glucose levels return to normal if they made changes to their daily habits. However, if nothing was done, elevated blood

glucose levels would in 40-50% the IGT subjects and an onset of diabetes occurred in the 10 years following.

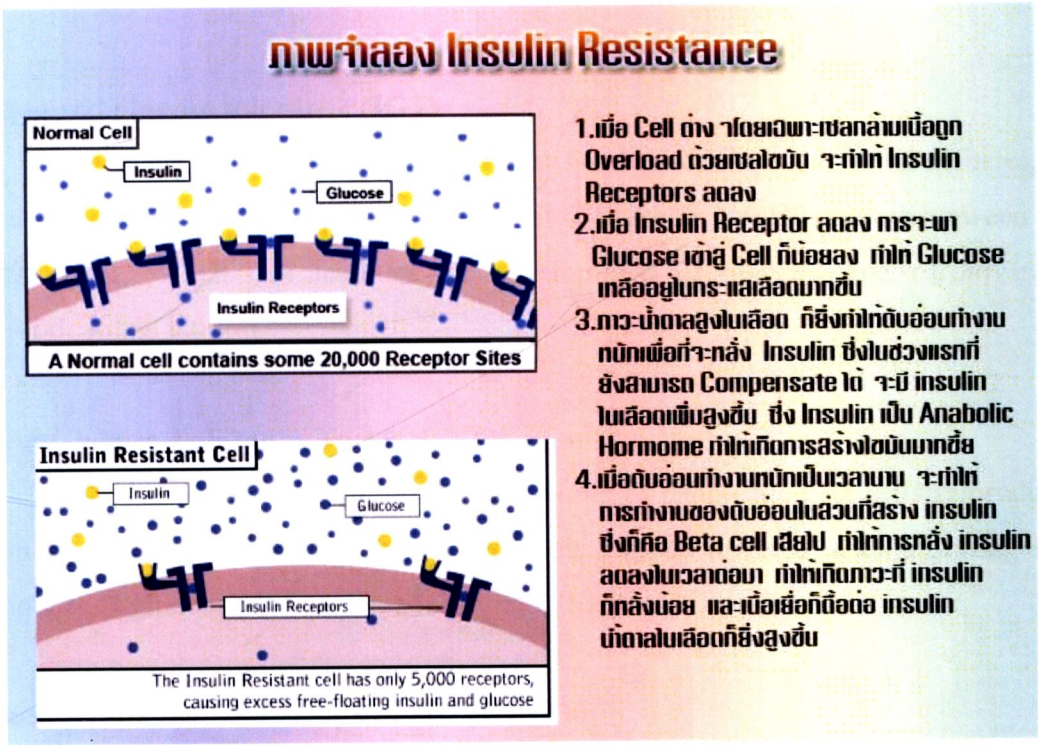

Figure 5 Model of insulin Resistance

Source: http://www.pcos.insulitelabs.com

1. When various cells, especially muscle cells are overloaded with intracellular fat, a decrease in insulin receptors occurs.

2. When there are less insulin receptors, there is less glucose uptake by the cells and as a result glucose will remain in the bloodstream.

3. The elevated blood glucose levels cause the pancreas to work hard and produce more insulin. In the initial stages, the pancreas is able to compensate and there is an elevated level of insulin in the blood. Since insulin is an anabolic hormone, production of fat occurs.

4. With prolonged hard work, beta cells in the pancreas begin to die, leading to a decrease in insulin production and tissues becoming insulin resistant; eventually higher levels of blood glucose is observed.

Impaired glucose tolerance (IGT)

IGT is glucose metabolic state that lies between normality and diabetes; that is a fasting glucose between 110-125 mg/dl or a 2-hour OGTT glucose between 140-199 mg/dl, which can lead to the development of diabetes and cardiovascular complications lateron.

Oral Glucose Tolerance Test (OGTT)

This test helps determine the functioning of pancreatic beta cells in producing insulin. In health individuals, glucose levels can be lowered after ingesting large amounts of glucose (75 grams or 1.75 grams/1 kg in children).

Homeostasis model assessment (HOMA) [30]

Equation to determine beta-cell function and insulin resistance:

$$\beta\text{- Cell function index} = \frac{20\text{x Fasting insulin } (\mu U / mL)}{\text{Fasting glucose } (\mu mol / L) - 3.5}$$

$$\text{Insulin resistance index} = \frac{\text{Fasting insulin } (\mu U / mL) \text{ x fasting glucose } (\mu mol / L)}{22.5}$$