CHAPTER III

METHODOLOGY

Research design: Apply research

Subjects

Thalassemia patients

     A total of 60 transfuse-dependent β-thalassemia patients, who had regularly received blood transfusion at the pediatrics and medical department, Vachira Phuket Hospital, were 26 males ages 5-18 years old and 34 females ages 5-19 years old.

Control subjects

     A 20 sex, age-matched normal subjects were included in the study as control group. This group consists of 10 males and 10 females, their ages were between 5-18 years old. All subjects had normal blood glucose levels and negative for hepatitis B and/or hepatitis C virus. Body weight, height, waist circumference, and blood pressure were recorded. Characteristics of patients and control subjects were given in Table 2. This study was approved by the ethical committee of Naresuan University and Vachira Phuket Hospital.

Table 2 Characteristics of study subjects

	Transfusion-dependent β-thalassemin patients	Control subjects
Age (years)	11.1* (8.0-15.7)**	10.8* (9.2-12.6)**
Males	26	10
Females	34	10

Note: *=Median, **= interquatile (Q1-Q3)

Sample collection

Blood samples were collected by vein puncture after an overnight fasting before blood transfusion on the transfusion-day. Blood was separated each 4 ml into sodium fluoride tube, EDTA tube and the other as clotted blood, respectively. Serum was separated by centrifugation and divided into 2 micro-tubes for the following purposes, blood chemistry parameters (Total Cholesterol (TC), Triglyceride (TG), HDL-C, LDL-C, AST, ALT and ALK), serum ferritin levels and hepatitis B, C virus, respectively. The EDTA blood was measured for complete blood count (Hb and Hct). All subjects will be tested for oral glucose tolerance test (OGTT) by drinking a 75 g of glucose (1.75g/kg in children) and after drinking blood will be drawn at 0.5, 1, 1.5 and 2 hr, were analyzed blood glucose levels. The other steps will progress after the 6 months interval, all transfuse-dependent β-thalassemia patients will be operate the same as in the first state before.

Analytical methods

Complete blood cell count

Whole EDTA- blood was initially taken for complete blood cell count analysis by using the ADVIA 120 autoanalyzer, Germany.

Biochemical test

All biochemical parameters (Glucose, BUN, Creatinine, Uric acid, TC, TG, High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), Aspartate transminase (AST), Alanine transminase (ALT), Alkaline phosphatase, Tatol bilirubin, Direct bilirubin,) were evaluated in serum by enzymetic on Olympus 400 and Olympus AU 640 autoanalyzer (E for L, japan)

Serum ferritin levels

Serum ferritin was measured by using ADVIA Centaur CP autoanalyzer, Germany

Insulin level

Insulin level was measured by using a microparticle enzyme immunoassay (MEIA), a commercial kit performed by Axsym autoanalyzer (Abbott diagnostic, USA).

Hepatitis B and Hepatitis C

Hepatitis B antigen (HBsAg) and Hepatitis C were measured by using a Microparticle Enzyme Immunoassay (MEIA), a commercial kit performed by AxSYM autoanalyzer (Abbott diagnostic, USA).

Statistical analysis

The results are expressed as the median and interquartile Q1-Q3 and compared two groups by Mann-Whitney U test for differences median. Spearman rank correlation test were used to determine relationships between covariates.