CHAPTER IV

RESULTS AND DISCUSSION

Demographic and clinical parameters of transfusion-dependent β-thalassemia patients and controls

All baseline characteristics of median and interquatile of age, height, weight, BMI and waist circumference (WC) of the 60 Transfusion-dependent **β-** thalassemia patients were demonstrated in Table 3. To account for sex and age-dependency, we converted the height and weight of the patients to mean from the actual measurement. In thalassemia patients were comprised matched normal subjects were included in the study as a control group.

Table 3 Demographic and clinical parameters of transfusion-dependent β-thalassemia patients and control

Parameters	β - thalassemia patients	Controls	*P*-value
Age (years)	11.1* (8.0-15.7)**	11.2 *(8.1-15.4)	0.541
Height (cm)	137.3 (119.2-154.8)	153.5 (145.8-161.2)	<0.001
Weight(kgs)	32.0 (20.1-43.9)	46.3 (42.0-50.6)	<0.001
BMI (kg/m2)	16.5 (13.6-19.3)	19.8 (18.3-21.3)	<0.001
WC (cm)	25.2 (22.8-28.4)	26.4 (24.7 -28.1)	0.100

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

Blood Analysis of the first period of this study

Blood concentrate

The hematocrit (Hct) and hemoglobin (Hb) values were listed in table 4 and compared with those of healthy controls. The hemoglobin and hematocrit concentrations were significantly lower in β-thalassemia patients than the normal controls as shown in table 4.

Table 4 Blood concentrate of the first patients and control

Parameters	β - thalassemia patients (1st)	Controls	*P*-value
Hb(gm/dl)	7.4 *(5.7-8.5)**	12.6* (11.8-14.5)**	<0.001
Hct (%)	23.2 (20.0-26.2)	38.3 (35.0-43.7)	<0.001

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

Biochemistry parameters

Blood for biochemistry parameters in the first period study, the baseline characteristics of β-thalassemia patients and control subjects were summarized in Table 5. TG, AST, ALT, ALT, ALP, total bilirubin, direct bilirubin, serum ferritin, insulin and IRI were significantly higher ($P<0.001$) than normal control subjects, especially the TG, AST, ALT, ALP, total bilirubin and direct bilirubin were two times higher than in control subjects. TC, HDL-C, and LDL-C were significantly lower ($P<0.05$) than normal controls. But in the FBS was significantly higher ($P<0.001$) than normal controls as shown in table 5.

The OGTT in β-thalassemia patients were significantly higher ($P<0.05$) only in 2.0 Hr as shown in table 5.

Table 5 Biochemical data of the first patient and control

Parameters	β - thalassemia patients (1st)	Controls	*P*-value
FBS (mg/dl)	82.0* (79.0-86.0)**	70.0* (69.0-71.0)**	<0.001
OGTT			
0.5 Hr Glu (mg/dl)	127.5 (119.0-143.0)	127.0 (123.4-131.8)	0.512
1.0 Hr Glu (mg/dl)	114.5 (106.3-127.0)	115.0 (110.0-119.8)	0.751
2.0 Hr Glu (mg/dl)	91.5 (85.0-101.0)	89.0 (83.3-91.8)	0.031
Cholesterol (mg/dl)	121.0 (98.2-135.0)	137.0 (130.5-146.7)	<0.001
Triglyceride (mg/dl)	123.0 (103.5-143.5)	55.5 (51.0-59.7)	<0.001
HDL-C(mg/dl)	32.0 (26.0-35.0)	35.0 (31.2-36.7)	0.016
LDL-C (mg/dl)	61.8 (49.0-74.5)	89.3 (86.7-97.3)	<0.001
AST (U/L)	40.0 (31.2-57.5)	14.0 (10.0-16.0)	<0.001
ALT(U/L)	32.0 (25.0-53.5)	12.0 (10.0-15.5)	<0.001
ALP(U/L)	120.0 (90.2-161.2)	54.5 (52.2-58.0)	<0.001
Total bilirubin (mg/dl)	2.0 (1.6-2.6)	0.2 (0.2-0.2)	<0.001
Direct bilirubin (mg/dl)	0.4 (0.4-0.6)	0.0 (0.0-0.1)	<0.001
Serum ferritin (ng/ml)	2,051.5 (1,405.0-2,958.0)	58.0 (48.2-70.0)	<0.001
Insulin (μU/ml)	15.1 (12.0-16.9)	8.8 (8.7-8.9)	<0.001
IRI	3.04 (2.36-3.56)	1.51(1.48-1.53)	<0.001

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

Blood Analysis of the second period after 6 moths in this study

Blood concentrate

The Hct and Hb values were listed in table 6 and compared with those of healthy controls. The Hct and Hb concentrations were significantly lower in β-thalassemia patients than the normal controls as shown in table 5.

Table 6 Blood concentrate of the second patients and control

Parameters	β - thalassemia patients (2^{nd})	Controls	*P*-value
Hb(gm/dl)	7.4* (5.9-8.0)**	12.6* (11.8-14.5)**	<0.001
Hct (%)	22.8 (19.4-26.0)	38.3 (35.0-43.7)	<0.001

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

Biochemistry parameters

Blood biochemistry parameters in the second period after 6 months in β-thalassemia patients and control subjects were summarized in Table 7. TG, AST, ALT, ALT, ALP, total bilirubin, direct bilirubin, serum ferritin, insulin and IRI were significantly higher ($P<0.001$) in β-thalassemia patients than normal controls, especially the TG, AST, ALT, ALP, total bilirubin and direct bilirubin were two times higher in β-thalassemia patients. TC, HDL-C, and LDL-C were significantly lower ($P<0.05$) in the β-thalassemia patients than normal controls. But in FBS level was significantly higher ($P<0.001$) in the β-thalassemia patients than normal controls as shown in table 7.

The OGTT were significantly higher ($P<0.05$) at 0.5, 1.0 and 2.0 Hr in β-thalassemia patients than normal subjects as shown in table 7.

Table 7 Biochemical data of the second patient and control

Parameters	β - thalassemia patients (2^{nd})	Controls	*P*-value
FBS (mg/dl)	84.0* (80.0-89.0)**	70.0* (69.0-71.0)**	<0.001
OGTT			
0.5 Hr Glu (mg/dl)	131.0 (125.0-140.8)	127.0 (123.4-131.8)	0.033
1.0 Hr Glu (mg/dl)	120.0 (113.3-128.9)	115.0 (110.0-119.8)	0.014
2.0 Hr Glu (mg/dl)	92.0 (85.0-101.8)	89.0 (83.3-91.8)	0.010
Cholesterol (mg/dl)	128.5 (114.0-143.7)	137.0 (130.5-146.7)	0.018
Triglyceride (mg/dl)	129.0 (110.2-139.0)	55.5 (51.0-59.7)	<0.001
HDL-C(mg/dl)	32.0 (27.7-35.0)	35.0 (31.2-36.7)	0.045
LDL-C (mg/dl)	72.2 (57.8-82.9)	89.3 (86.7-97.3)	<0.001
AST (U/L)	38.0 (30.0-54.0)	14.0 (10.0-16.0)	<0.001
ALT(U/L)	35.5 (25.0-55.0)	12.0 (10.0-15.5)	<0.001
ALP(U/L)	125.5 (95.2-161.)	54.5 (52.2-58.0)	<0.001
Total bilirubin (mg/dl)	1.9 (1.6-2.5)	0.2 (0.2-0.2)	<0.001
Direct bilirubin (mg/dl)	0.4 (0.4-0.6)	0.0 (0.0-0.1)	<0.001
Serum ferritin (ng/ml)	1,997.5 (1,429.5-2,832.2)	58.0 (48.2-70.0)	<0.001
Insulin (μU/ml)	16.3 (12.6-17.7)	8.8 (8.7-8.9)	<0.001
IRI	3.35 (2.58-3.80)	1.51(1.48-1.53)	<0.001

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

In the study result was not clear in the interpretation of impaired fasting blood glucose or OGTT test. Only at 0.5, 1.0 and 2.0 Hr of OGTT test were significantly higher ($P<0.05$) in β-thalassemia patients than normal subjects. But the insulin concentration results indicated that the thalassemic patients were showed higher than 10 μU/ml. These may indicate the stage of these thalassemic patients were hyperinsulinemia or insulin resistance status (table 5, 7).

Comparison of all parameters in first and second period in this study

Blood concentration

The Hb levels were no significantly difference, but Hct levels were significantly higher in the first period than second period in β-thalassemia patients as shown in table 8.

Table 8 Blood concentrate of the first patients and the second patients

Parameters	β - thalassemia patients (1^{st})	β - thalassemia patients (2^{nd})	*P*-value
Hb(gm/dl)	7.4* (5.7-8.5)**	7.4* (5.9-8.0)**	<0.360
Hct (%)	23.2 (20.0-26.2)	22.8 (19.4-26.0)	<0.049

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

Biochemistry parameters in the first and second period study

Blood biochemistry parameters in the first period study were compared with the second period study in β-thalassemia patients. TC, LDL-C, Insulin, IRI TG, HDL-C and ALT were significantly higher ($P<0.05$) in second period than the first period in β-thalassemia patients. In AST, ALP, total bilirubin, direct bilirubin and serum ferritin were not significant differences ($P>0.05$) in the first and second period study. The FBS level was significantly higher ($P<0.001$) in the second period than second period study.

The OGTT at 0.5 and 1.0 Hr of the second period were significantly higher ($P<0.05$) than first period study, at 2.0 Hr. was not significant differences ($P>0.05$).

Table 9 Comparison of the biochemical levels in the first and second period study

Parameters	β - thalassemia patients (1st)	β - thalassemia patients (2nd)	*P*-value
FBS (mg/dl)	82.0* (79.0-86.0)**	84.0* (80.0-89.0)**	<0.001
OGTT			
0.5 Hr Glu (mg/dl)	127.5 (119.0-143.0)	131.0 (125.0-140.8)	0.008
1.0 Hr Glu (mg/dl)	114.5 (106.3-127.0)	120.0 (113.3-128.9)	0.010
2.0 Hr Glu (mg/dl)	91.5 (85.0-101.0)	92.0 (85.0-101.8)	0.074
Cholesterol (mg/dl)	121.0 (98.2-135.0)	128.5 (114.0-143.7)	<0.001
Triglyceride (mg/dl)	123.0 (103.5-143.5)	129.0 (110.2-139.0)	0.008
HDL-C(mg/dl)	32.0 (26.0-35.0)	32.0 (27.7-35.0)	0.007
LDL-C (mg/dl)	61.8 (49.0-74.5)	72.2 (57.8-82.9)	<0.001
AST (U/L)	40.0 (31.2-57.5)	38.0 (30.0-54.0)	0.138
ALT(U/L)	32.0 (25.0-53.5)	35.5 (25.0-55.0)	0.033
ALP(U/L)	120.0 (90.2-161.2)	125.5 (95.2-161.)	0.785
Total bilirubin (mg/dl)	2.0 (1.6-2.6)	1.9 (1.6-2.5)	0.742
Direct bilirubin (mg/dl)	0.4 (0.4-0.6)	0.4 (0.4-0.6)	0.075
Serum ferritin (ng/ml)	2,051.5 (1,405.0-2,958.0)	1,997.5 (1,429.5-2,832.2)	0.163
Insulin (μU/ml)	15.1 (12.0-16.9)	16.3 (12.6-17.7)	<0.001
IRI	3.04 (2.36-3.56)	3.35 (2.58-3.80)	<0.001

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

The correlation of FBS with other parameters

The FBS level was significantly correlated with age, BMI, WC, serum feritin, insulin and IRI (r=0.457 (*P*<0.001), r=0.318 (*P*=0.013), r=0.401 (*P*=0.001), r=0.314 (*P*=0.015), r=0.719 (*P*=<0.001) and r=0.840 (*P*<0.001, respectively) as shown in Table 10.

Table 10 The correlation of FBS with other parameters

Parameters	Correlations	*P*-value
Age (years)	0.457	<0.001
BMI (kg/m2)	0.318	0.013
Waist (cm)	0.401	0.001
Serum ferritin (ng/ml)	0.314	0.015
Insulin (μU/ml)	0.719	<0.001
IRI	0.840	<0.001

The correlation of Total Cholesteral with other parameters

The TC level was significantly correlated with ALT (r=0.328, *P*=0.010) as shown in Table 11.

Table 11 The correlation of total cholesteral with other parameters

Parameters	Correlations	*P*-value
ALT	0.328	0.010

The correlation of Triglyceride with other parameters

The TG level was significantly correlated with AST and ALT (r=0.337, *P*=0.008 and r=0.489, *P*<0.001, respectively) as shown in Table 12.

Table 12 The correlation of triglyceride with other parameters

Parameters	Correlations	*P*-value
AST	0.337	0.008
ALT	0.489	<0.001

The correlation of serum ferritin with other parameters

The serum ferritin level was significantly correlated with AST and ALT (r=0.500, $P<0.001$ and r=0.473, $P<0.001$, respectively) as shown in Table 13.

Table 13 The correlation of Serum ferritin with other parameters

Parameters	Correlations	*P*-value
AST	0.500	<0.001
ALT	0.473	<0.001

Discussion

In this study found that β - thalassemia patients who received the blood transfusion, had body component such as height, weight and BMI were lower than normal controlled group. The development of their bodies did not agree with ages. The serum ferritin level in the β - thalassemia patients were significantly higher than normal controls showed that these β - thalassemia patients were in the iron overload condition. The higher in ferritin levels mean the over collection limit of iron [31], as in the results of serum ferritin level in the β - thalassemia patients was higher 35 fold than in normal controls (as 2,051.5 ng/ml in β - thalassemia patients and 58.0 ng/ml in normal controls). All most of these β - thalassemia patients never received the iron chelating drug, such as deferoxamine (DFO). Only 5 cases ever have received, by subcutaneous infusion but not regularly. Some cases have taken during 1-2 years ago. This result similar to the study of Geoge, et al.(1994) found that 24 volunteer thalassemia patients had increased the level of serum ferritin more than the average mean 2,066.7 ng/ml ± 1,265.3 was 5,557 ng/ml ± 2,041.4 within the 3 years of study research [31], Livrea, et al. had studied 25 volunteer patients found that the level of

serum ferritin higher than normal volunteer 20 times (1,866 ng/ml ± 996). Every patient has received deferoxamine (DFO) at least 5 times in a week by subcutaneous infusion 8-12 hours [32], so that the level of serum ferritin was lower than current study research.

From iron overload, the level of serum ferritin had so much increased showed that the body's situation might caused stimulate reaction of Fenton and Harber Weiss then changed to superoxide radical or $O_2\cdot$ and hydrogen peroxide or H_2O_2 to be Hydroxyl radical or ·OH reaction.

$$H_2O_2 + O_2^{\cdot} + Fe^{2+} \longrightarrow OH + \cdot OH + Fe^{3+} \text{ .....Harber-Weiss reaction}$$

$$H_2O_2 + Fe^{2+} \longrightarrow OH + \cdot OH + Fe^{3+} \text{.....Fenton reaction}$$

OH radical will lead to be lipid peroxidation of plasma membrane such as mitrochondia, lysosomes and sacroplasmic membranes. There were evidences of tissue had been destroyed found in the experimental human bodies and the animal bodies which were in the condition of iron overloading and in the thalassemia patients. [24] The patients with iron overloaded, protein transferin will be saturated could not mix up to each others (Nontransferin-bound iron: NTBI) had been found in plasma. [25] NTBI might be a radical stimulator Hydroxyl [26] and increase iron absorption with in tissue. [27] There were increasing evidences of mini iron chemical compound in serum and in cell. These stimulated conditions caused the demolition of plasma membrane and cells that have overloaded of iron. Liver, pituitary gland, pancreas and other cells, if pancreas were destroyed may caused lacking of hormone insulin which was created from cells in pancreas. [4] Abnormal of glucose metabolism would caused diabetes.

From this study found that the β-thalassemia patients had FBS higher than normal controlled group in two times. And from the OGTT test found that glucose level no significance different when compared to the patients from the first time and second time still found that between 0.5 and 1 Hr had higher level in the second time with significance statistic. In the second time point 2 Hr. found that there was not any significance statistic. Which showed that the β-thalassemia patients who had to receive

blood transfusion might have trend to be higher glucose and impaired glucose tolerance, but may need more time or growing age or may use other markers that could have better follow up. This study had patient found that there were impaired glucose toleranace only 1 case from 60 cases, about 1.67%. Which were different from the result of Jaruratanasirikul, et al. [30] 12.5 % between the age 16.5±3.4 years, found that higher age interval more than this study. From the analysis level of insulin that pointed out more accurately than OGTT. Then we know, β - thalassemia patients had hyperinsulinemia which might be insulin resistant but the body could compensated. This insulin level could lead glucose to help metabolism in cell, and diabetes or impaired glucose tolerance would never happened. (Table 7, 8, 9)

From correlation of FBS and level of Serum ferritin which are in the same direction. Increasing of Serum feritin caused level of FBS increased. Correlation r = 0.314 (P=0.015), when sugar increased, pancreas will produce more Insulin in order to lead sugar in the blood circulation into cells from correlation r = 0.719 (P<0.001) but sugar level still being high, it might caused anti-insulin from correlation r = 0.840 (P<0.001).

From the study found that level of Cholesterol, HDL-C and LDL-C were clearly lower than normal volunteer. There were study showed that Oxidative stress oxidized LDL-C molecule became Oxidize-LDL and might caused foam cells and Atherosclerosis or blood clotting and cardiovascular disease which were the main diseases of thlassemia patients death. [28]

From correlation of Cholesterol level and ALT r = 0.328 (P=0.010) and correlation of Triglyceride with AST and ALT r = 0.500 (P<0.010) and r = 0.473 (P<0.001) respectively came from cells were destroyed.

Condition of iron could help to stimulate liver diseases and were the cause of death which always happened to the transfusion-dependent β - thalassemia patients within 2 years after started to receive blood, found collagen formation and portal fibrosis in cases did not chelating therapy [33]

To adjust a suitable iron density of β - thalassemia patients, controlling quantity of iron must not be over 15 mg/g liver which might cause heart diseases and die.

In case of iron quantity at the level of 7-15 mg/g liver, it would have affected to the complexity from iron overload such as; Hepatic fibrosis. And in this level, giving anti iron is necessary to the treatment but every case of the overloaded 15 mg/g liver must be chelated. [34]

Serum ferritin level was used to evaluate iron quantity in the body direct and indirect. Because could not use the process of analysis to evaluate iron quantity in the body. Analysis quantitative of ferritin in serum or plasma was the method that evaluates iron quantity in the body [35]. Ferritin level, the result might not directly to the iron quantity in the body in some cases such as: ascorbate deficiency, fever, acute infection, chronic inflammation, acute and chronic hepatic damage, hemolysis and ineffective erythropoiesis [36] Similarly level of serum iron, transferin, transferin saturation and density of transferin receptor or urinary iron which were driven out with urine while received iron chelating DFO, did not reflect to iron quantity in the body. The result to find analysis process that can reflect iron quantity exists in the body that would be useful for easy analysis and follow up NTBI level but still not generally use.