RESEARCH ARTICLE

Glucose and Lipid Profiles in Adolescents with Thalassemia Major and Its Association with Iron Overload in Specific Organs

Aryono Hendarto[1,*], Teny Tjitra Sari[2], Ludi Dhyani Rahmartani[2], Anggia Widyasari[3], Stephen Diah Iskandar[3]

[1]Division of Pediatric Nutrition and Metabolic Disease, Department of Child Health, Dr. Cipto Mangunkusumo Hospital - Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.5, Jakarta
[2]Division of Pediatric Hematology Oncology, Department of Child Health, Dr. Cipto Mangunkusumo Hospital - Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.5, Jakarta
[3]Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo Hospital, Jl. Salemba Raya No.5, Jakarta

*Corresponding author. E-mail: aryhendarto@yahoo.com

Received date: Dec 25, 2018; Revised date: Feb 26, 2019; Accepted date: March 4, 2019

Abstract

BACKGROUND: Organ damage due to iron toxicity is one factor that increases the risk of getting cardiovascular and metabolic diseases in thalassemia patient. This study aims to determine glucose and lipid profiles in adolescents with thalassemia major and its association with iron overload in pancreas and liver.

METHODS: This was a cross sectional study. Subjects were thalassemia major adolescents without any confounding factors that may affect glucose and lipid levels. Blood samples were collected to measure the glucose level, lipid profiles, ferritin level and transferrin saturation. T2-Magnetic Resonance Imaging was used to evaluate the iron overload in organs.

RESULTS: From a total of 60 subjects, diabetes mellitus was diagnosed in 1 subject and impaired fasting glucose was diagnosed in 3 subjects. All subjects had high triglycerides/high density lipoprotein-cholesterol (HDL-C) ratio, 59 subjects (98%) had low HDL-C, 18 subjects (30%) had hypertriglyceridemia, and none had abnormal high level of low density lipoprotein-cholesterol (LDL-C). The majority of subjects had ferritin ≥2,500 ng/mL (70%), mild pancreatic iron overload (56.6%), and moderate hepatic iron overload (43.8%). Degree of hyperferritinemia was not associated with glucose and lipid profiles. Blood glucose profiles were not associated with various degree of pancreatic iron overload. Similar result was also observed between lipid profiles and hepatic iron overload.

CONCLUSION: Abnormal glucose and lipid profiles in thalassemia major can be found in adolescence. Normal blood glucose level isn't necessarily associated with normal pancreatic iron deposition. Hepatic iron overload may worsen dyslipidemia in thalassemia major patients.

KEYWORDS: glucose profile, lipid profile, pancreatic iron overload, hepatic iron overload, thalassemia major

Indones Biomed J. 2019; 11(2): 188-93

Introduction

Thalassemia is an autosomal recessive disorder, characterized by decrease or absence in production of globin chain.(1) As the most common single gene disorder, around 300 mutations in β-globin gene and 100 mutations in α-globin gene have been found around the world.(2) Patient with homozygous or severe mutation usually presents as thalassemia major, indicated by the need of life-long transfusion to correct severe anemia.(3)

The most common complication related to regular transfusion is iron overload.(3) Liver and pancreas are two organs with high iron storage capacity.(4) These two organs play an essential role in metabolism. The liver regulates cholesterol metabolism and the pancreas controls blood glucose levels by producing insulin hormone and its counter-regulatory hormone glucagon.(5-7)

In the last decades, thalassemia major patients tend to have better survival rate as a result of novel therapeutic and complications prevention strategies. Those strategies include the improvement of blood quality, invention of new oral iron chelator, better adherence with iron chelation treatment, therapy starting at earlier age, and availability of T2-Magnetic Resonance Imaging (MRI) to assess degree of iron deposition in organs.(8,9) With this prolonged survival, chronic complications in thalassemia major patients, such as metabolic syndrome and cardiac disease, cannot be avoided.

Many pathogeneses of chronic disease, such as atherogenesis and insulin resistance, have started since the adolescence or earlier period in life.(10-12) Therefore, detecting metabolic abnormalities at a young age is very important. We are interested in looking upon glucose and lipid profiles in adolescent thalassemia major patient because there are still limited studies available in this field. We also aimed to determine the association between glucose profiles with pancreatic iron overload, as well as lipid profiles with hepatic iron overload.

Methods

This was a cross sectional study conducted at Cipto Mangunkusumo Hospital, Jakarta, Indonesia. This study had been approved by ethical committee of Faculty of Medicine, Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta (No. 1113/UN2.F1/ETIK/2018).

We estimated the sample size using sample estimation formula for measuring mean difference between two groups, with α=0.05 and β=0.8. Pooled estimate of the variance was obtained from previous researches.(13-14) Mean glucose and cholesterol levels were assumed as clinically significant if the differences were 10 mg/dL and 20 mg/dL, respectively. A minimum of 60 adolescents (age 12-18 year) with thalassemia major must be recruited for this study. Subjects were excluded from the study if they were on diet program or consumed certain drugs, including antidiabetic, antiobesity, and steroid drugs.

Pre-transfusion fasting blood samples were collected to measure fasting blood glucose, lipid profiles (triglyceride, total cholesterol, high density lipoprotein-cholesterol (HDL-C), and high density lipoprotein-cholesterol (LDL-C), ferritin and transferrin saturation. After that, subject was given 75 g glucose to measure 2-hour post-load blood glucose (2hPG) level.

Hyperferritinemia was categorized into two groups: 1,000-2,500 ng/mL and ≥2,500 ng/mL.(15) Diabetes mellitus was defined by fasting plasma glucose (FPG) level ≥126 mg/dL or 2hPG level ≥200 mg/dL. Isolated impaired fasting glycaemia (IFG) was defined by FPG level 100-125 mg/dL and 2hPG level <140 mg/dL. Isolated impaired glucose tolerance (IGT) was defined by FPG level <100 mg/dL and 2hPG level 140-199 mg/dL.(16-18) Hypertriglyceridemia was defined by a cut-off ≥150 mg/dL.(16) High LDL-C and triglyceride/HDL-C were defined by a level ≥130 mg/dL and ≥2.2, respectively.(19,20) Low HDL-C was defined by a value <40 mg/dL.(21)

Degree of iron deposition in pancreas and liver was assessed using T2-MRI 1.5 Tesla (CMRtools, Siemens Avanto, Erlangen, Germany). Degree of pancreatic iron deposition was classified as follow: normal >33 ms, mild 10-33 ms, moderate 2.5-10 ms, severe <2.5 ms. Degree of hepatic iron deposition was classified as follow: normal >6.3 ms, mild 2.7-6.3 ms, moderate 1.4-2.7 ms, severe <1.4 ms.(22,23)

Data results were presented as numeric data and analyzed using SPSS 20.0 (IBM, Chicago, USA). The relationship between degree of hyperferritinemia with glucose and lipid level were analyzed using unpaired t-test analysis. Meanwhile, data analysis between iron overload in organs (based on T2-MRI) with glucose and lipid level were performed with Kruskal-Wallis.

Results

A total of 60 subjects were recruited in this study. The number of male and female subjects were almost equal. Most of the subjects were diagnosed with β-thalassemia (51.7%) and β-thalassemia/HbE (45%). Deferiprone (DFP) was the most common iron chelator, either as single therapy or combined with deferasirox (DFX) . None of them used deferoxamine (DFO) as single iron chelator (Table 1).

One subject was diagnosed with diabetes mellitus and 3 subjects were diagnosed with IFG. Subject with diabetes mellitus was 16 years old boy. He had been diagnosed with beta-thalassemia major when he was 19-months old. He used single DFP iron chelator with dosage of 75 mg/kg body weight/day. There were 18 subjects diagnosed with hypertriglyceridemia, but none with high LDL-C. Most subjects had low HDL-C and high triglyceride/HDL-C ratio. Based on serum ferritin level, most of the subjects (70%) had ferritin level ≥2500 ng/mL. T2-MRI examination showed that subjects predominantly had mild pancreatic iron overload (56.6%) and moderate hepatic iron overload (43.8%) (Table 2).

Table 1. Demographic characteristics of subjects.

Variables	n = 60
Age, mean (SD) years	15 (1.89)
Gender, n (%)	
Male	32 (53.3)
Female	28 (46.7)
Type of thalassemia, n (%)	
α-thalassemia	2 (3.3)
β-thalassemia	31 (51.7)
β-thalassemia/HbE	27 (45)
Type of chelation, n (%)	
DFP	31 (51.7)
DFX	6 (10)
DFO+DFP	8 (13.3)
DFO+DFX	1 (1.7)
DFP+DFX	14 (23.3)

DFP: deferiprone; DFX: deferasirox; DFO: deferoxamine.

In general, the degree of hyperferritinemia did not significantly affect glucose and lipid profiles (Table 3). Glucose profiles were not related with the degree of pancreatic iron overload (Table 4).

The degree of hepatic iron overload was not associated with lipid profiles. Following the increased severity of hepatic iron overload, the trend of triglyceride level and triglyceride/HDL-C ratio were positive, but the trend of total cholesterol, HDL-C, and LDL-C levels were negative (Table 5).

Discussion

Iron overload is one of the main complications in thalassemia major patients. It induces cell death and organ damages by generating reactive oxygen species (ROS). Evaluating iron overload in organs is crucial for thalassemia major patients. In our study, we found that most adolescents with thalassemia major had mild pancreatic iron overload and moderate hepatic iron overload. Another study observed that iron deposition in thalassemia major children has occurred before adolescence.(4)

Serum ferritin level was not associated with glucose and lipid levels. It may be explained by the fact that serum ferritin is sensitive, but not specific to express iron level in the body.(24) Many factors may affect serum ferritin level, including dietary intake of iron-rich products, obesity, inflammation, liver disease, and alcohol consumption.(25) Therefore, T2-MRI examination is essential to directly measure iron deposition in organs.(4)

In this study, we diagnosed 1 subject with diabetes mellitus, 3 subjects with IFG, and none with IGT. These findings indicate that the abnormality of glucose metabolism in thalassemia major has started early in life. On the contrary, MRI examination showed that most subjects had abnormal pancreatic iron deposition. This

Table 2. Glucose profile, lipid profile and iron overload in organs.

Variables	n = 60
Glucose tolerance test, mean (SD) mg/dL	
FPG	90.14 (22.72)
2hPG	98.78 (19.72)
Glucose abnormalities, n (%)	
Diabetes mellitus	1 (1.7)
Isolated IFG	3 (5)
Lipid profile, mean (SD) mg/dL	
Triglyceride	143.16 (50.02)
Total-C	94.24 (22.41)
HDL-C	20.70 (7.53)
LDL-C	54.22 (17.49)
Triglyceride/HDL-C ratio	7.85 (4.63)
Lipid abnormalities, n (%)	
Hypertriglyceridemia	18 (30)
High triglyceride/HDL-C	60 (100)
Low HDL-C	59 (98)
Ferritin, median (min-max) ng/mL	3565 (1013 - 14126)
Transferrin saturation, median (min-max) %	89.5 (30 - 100)
Degree of hyperferritinemia, n (%)	
1,000-2,500 ng/mL	18 (30)
≥2,500 ng/mL	42 (70)
Pancreatic iron overload, n (%)	
Normal	13 (21.7)
Mild	34 (56.6)
Moderate	13 (21.7)
Hepatic iron overload, n (%)	
Normal	3 (4.2)
Mild	15 (25)
Moderate	26 (43.8)
Severe	16 (27)

FPG: fasting plasma glucose; 2hPG: 2-hour post-load blood glucose; IFG: impaired fasting glycaemia; Total-C: total cholesterol; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol.

Table 3. Blood glucose and lipid profile level in different degree of iron overload.

Hyperferritinemia	FPG	2hPG	Triglyceride	Total-C	HDL-C	LDL-C	Triglyceride/ HDL-C Ratio
1,000-2,500 ng/mL	89.00 ± 9.97	96.53 ± 15.05	132.27 ± 25.47	90.33 ± 15.25	20.47 ± 5.62	52.13 ± 10.62	6.99 ± 2.25
≥2,500 ng/mL	90.61 ± 9.24	99.72 ± 21.49	150.39 ± 58.13	97.83 ± 24.27	21.22 ± 8.4	56.42 ± 19.68	8.25 ± 5.36
p -value*	0.596	0.550	0.088	0.08	0.509	0.165	0.766

**p*-value tested with unpaired t-test. FPG: fasting plasma glucose; 2hPG: 2-hour post-load blood glucose; Total-C: total cholesterol; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol.

discrepancy was also observed by other studies.(26,27) These results may imply two possibilities; firstly, despite damaged of pancreas caused by iron excess, production of pancreatic hormones is still normal. Another possibility is the compensatory mechanism in human body maintaining glucose homeostasis even when the production of pancreatic hormones has decreased. Further studies including the direct measurement of insulin level are important to explain these findings.

Long term glycemic monitoring is also important in thalassemia major. Two indicators that have been used in some studies are HbA1C and fructosamine. Fructosamine, a product resulted from glycation reaction between glucose and protein, is believed to be useful in patients with hemolytic anemia or hemoglobinopathies. However, studies comparing these markers show inconsistent results.(28,29) Liver iron overload in thalassemia major patients causes impairment on its function.(30,31) Theoretically, liver plays an important role in production, storage, and metabolism of lipid and lipoprotein in the body. One study finds that HDL-C and LDL-C levels are significantly affected by the severity of liver injury in non-alcoholic liver cirrhosis.(32)

In this study, we found that dyslipidemia in thalassemia major patients, especially high triglyceride/ HDL-C ratio and low HDL-C level, had started at early age. Hypocholesterolemia in thalassemia patients may be caused by cytokines release from macrophage system that impact hepatic secretion. Another factor is increased cholesterol uptake by macrophage to be used as membrane cell component during accelerated erythropoiesis in reticuloendothelial system.(33-35)

Despite having low LDL-C level, all subjects had high triglyceride/HDL-C ratio. This ratio is a good predictor for cardiometabolic risk (CMR) and insulin resistance in children.(36,37) It is inversely related to large LDL-C concentration and positively related to small LDL-C concentration.(38) Although all subjects in our study had normal level of LDL-C, but its proportion was dominated by small dense LDL-C. Previous study had proven that the small dense LDL-C has the highest atherogenic potential among lipoproteins in body.(39) In addition, nearly all subjects had low HDL-C level, which indicates impairment of scavenger mechanism to remove cholesterol and phospholipid molecules from cells and hence increases risk of atherosclerosis.(40)

We observed specific pattern of lipid profiles following increase in severity of hepatic iron overload, although the association between them was not significant. Triglyceride level and triglyceride/HDL-C ratio increased following the severity of hepatic iron overload. Meanwhile, HDL and LDL cholesterol level decreased, with the lowest level found in subjects with severe hepatic iron overload. Hepatic iron toxicity causes a decrease of apolipoprotein A and B production.(35) Therefore, hepatic iron toxicity should be

Table 4. Blood glucose level in various degree of pancreatic iron deposition.

Pancreatic Iron Overload	FPG		2hPG	
	Mean ± SD (mg/dL)	*p* -value*	Mean ± SD (mg/dL)	*p* -value*
Normal	91.50 ± 3.59		98.80 ± 24.54	
Mild	87.19 ± 8.94	0.071	96.12 ± 17.44	0.703
Moderate	94.20 ± 8.54		105.40 ± 23.36	

**p*-value tested with Kruskal Wallis, comparison between normal, mild and moderate group. FPG: fasting plasma glucose; 2hPG: 2-hour post-load blood glucose;

Table 5. Lipid profiles level in various degree of hepatic iron deposition.

Hepatic Iron Overload	Triglyceride		Total-C		HDL-C		LDL-C		Triglyceride/HDL-C	
	Mean ± SD (mg/dL)	*p* -value*	Mean ± SD (mg/dL)	*p* -value*	Mean ± SD (mg/dL)	*p* -value*	Mean ± SD (mg/dL)	*p* -value*	Mean ± SD (mg/dL)	*p* -value*
Normal	140.50 ± 28.99	0.991	98.50 ± 31.82	0.384	28.00 ± 11.31	0.065	55.00 ± 16.97	0.357	5.23 ± 1.08	0.327
Mild	134.92 ± 21.72		102.58 ± 30.70		23.33 ± 8.61		60.42 ± 23.02		6.43 ± 2.17	
Moderate	144.09 ± 42.37		95.38 ± 19.89		21.19 ± 8.50		54.10 ± 16.82		7.79 ± 3.64	
Severe	157.77 ± 82.31		83.54 ± 14.23		16.69 ± 3.23		46.54 ± 11.54		10.33 ± 4.48	

**p*-value tested with Kruskal Wallis, comparison between normal, mild, moderate and severe group.Total-C: total cholesterol; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol.

treated to prevent further decrease of HDL-C level and subsequent increase of triglyceride/HDL-C level.

A nutritionally balanced food with micronutrients supplementation, except iron, is strongly suggested for thalassemia patients. Diet modification may be helpful to alleviate metabolic problems in thalassemia major patients. Since cholesterol level in thalassemia patients is low, low carbohydrate diet may be offered if triglyceride level is high.(41,42)

Further researches with higher number of patients, controlled subject's diet, and more specific and sensitive markers are needed to evaluate metabolism abnormalities in thalassemia major patients.

Conclusion

Abnormalities of glucose and lipid metabolism in thalassemia major patients has developed since adolescence. Pancreatic iron overload may have existed before abnormalities of glucose metabolism is detected. Hepatic iron overload may worsen dyslipidemia in thalassemia major patients.

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