Correlation between Hepcidin and Ferritin with Insulin and Hba1C as Biochemical Markers of Pancreas Damage in β-Thalassemia Patients

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Abstract: Iron overload in β-thalassemia patients can damage various organs, including the pancreas. Impairment of the pancreas will be causing the failure and reduces insulin secretion, contributing to impaired glucose metabolism to diabetes mellitus in patients with β-thalassemia. This study analysed the correlation between hepcidin and ferritin levels as markers of iron overload with insulin levels and HbA1c as markers of pancreatic damage in patients with β thalassemia. Subjects of 35 thalassemia β patients were included in a cross-sectional study. Hepcidin, insulin, and HbA1c data were measured using the ELISA method. Ferritin data obtained through patient medical records. Bivariate analysis is using the Pearson test.: There were 97.1% of subjects with low hepcidin levels, whereas the ferritin data were in the high category. The data showed that 88.6% of subjects had low insulin levels. Most of the subjects (85.7%) were in the low category of HbA1c levels. Pearson test had a p-value = 0.001 which it indicates a significant relationship between hepcidin and insulin (r=0.771) and HbA1c (r=0.849). However, the ferritin levels showed no significant relationship with insulin (p=0.785 ; r=0.057) and HbA1c (p=0.420 ; r=0.169). In conclusion, the study found the lower the hepcidin level, the lower the insulin and HbA1c levels. Ferritin levels do not have a significant relationship with insulin and HbA1c levels.

1 INTRODUCTION

Thalassemia is a hereditary disorder syndrome caused by mutations in the globin gene that decrease or do not produce one or more globin chains (Origa, 2017; Soteriades and Weatherall, 2014). The International Thalassemia Foundation data shows that 7% of the world's population are carriers of thalassemia traits, and most of them are in developing countries. Indonesia, one of the endemic countries, has a high frequency of the β thalassemia gene with a rate of 3-10% (Rujito et al., 2015).

β thalassemia patients have decreased β globin chain synthesis, leading to a faster hemolysis process. Patients then require repeat transfusions every 4-6 weeks to maintain total erythrocytes and haemoglobin levels than 10g/dL. However, these long-term transfusions can cause iron overload (Vasudev and Sawhney, 2014). Hepcidin, the main protein acting as a negative regulator of iron, decreased due to ineffective erythropoietic activity. The decreased hepcidin levels can increase iron absorption in the intestine and the release of iron by macrophages, leading to the burden of iron overload in patients with β thalassemia (Jones et al., 2015).

The iron overload condition tends to an increase in ferritin levels. The increased ferritin levels exceed their capacity to bind iron that can cause an increase

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in free iron levels. The increase in free iron levels through the Fenton reaction will produce free radicals such as hydroxyl radicals (OH-) and hydroxyl anions (OH *), which can oxidise lipid components, denaturation proteins, and damage cell DNA replication (He et al., 2016; Maslowska, Makiela-Dzbenska and Fijalkowska, 2019). The pancreas also one of the organs which be affected and at risk of damage. Free radicals in the pancreas cause β cell death, impairing insulin secretion, which contributes to impaired glucose metabolism to diabetes mellitus in thalassemia patients (Nakavachara et al., 2020).

HbA1c is an indicator that can assess pancreatic function by measuring the average blood glucose level for three months (Gupta, Jain, and Chauhan, 2017). Evaluation of the iron overload condition in thalassemia patients can help the clinician to predict the damage on various organs, including the pancreas. This study aimed to determine the relationship between hepcidin and ferritin levels as markers of iron overload conditions with insulin and HbA1c levels as biochemical markers of pancreatic damage in β thalassemia patients.

2 MATERIALS AND METHODS

The study used an analytic observational with a cross-sectional study to determine the role of hepcidin and ferritin in pancreatic damage due to iron overload in patients with β thalassemia. The sampling method was a total sampling using 35 β-thalassemia patients registered in the parent's association of Indonesian thalassemia (POPTI) Samarinda East Kalimantan in 2019 had signed the consent form.

Hepcidin, insulin, and HbA1c examination were performed using the enzyme-linked immunosorbent assay (ELISA) sandwich method at the Research Laboratory of the Faculty of Medicine, Jenderal Soedirman University using BT Technology Elisa Kit manufacturer. Technical analysis for detection used the ELISA reader 270 Biomerieux, while the software used was https://www.elisaanalysis.com available on the online platform. In summary, the standards and samples were pipetted to the well coated with the antibody. The primary antibody was then added, followed by incubation. Secondary antibody and chromogen substance are added. The colour formed was then read in the absorbance value at the 450 nm. At the same time, Ferritin data were obtained from patient medical records.

Ethics approval came from the Health Research Ethics Commission, Faculty of Medicine, Jenderal Soedirman University. The Shapiro-Wilk test analysed numerical data distribution, while the analysis between variables was using the Pearson correlation on IBM SPSS Statistics 26 software.

3 RESULTS

Tables depicted the data from 35 patients with β-thalassemia, as shown in Table 1, Table 2. Most of the subjects were adolescence (48.6%) and children 40%). Only several subjects were below five years old and the elderly.

The number of male and female subjects was not much different. Most of the subjects' hepcidin levels were in a low category (97.1%) with a median value of 0.19 (0.12-2.55) ng/mL. Data on ferritin levels in research subjects only obtained 25 patients from a total of 35 patients, and all were in the high category with a median value of 3283 (1059-9748) ng/mL. Ferritin, at this point, were collected and averaged using three sequential times for recent measurement. The subjects' insulin levels had a median value of 2.19 (1.19-60.83) µU/mL, and most of them were in a low category (88.6%). Patients who have lower HbA1c levels (85.7%) were more than subjects who had an average (11.4%) and high (2.9%) levels with a median value of 26.95 (16.37-159.39) mg/dL.

Table 1. Characteristics of Respondents

Variable		Frequency	
		N	%
Age	<5years old	1	2,9
	5-11years old	14	40
	12-25 years old	17	48,6
	26-45 years old	2	5,7
	>45 years old	1	2,9
	Total	35	100
Gender	Male	17	48,6
	Female	18	51,4
	Total	35	100
Hepcidin	Low (<2ng/ml)	34	97,1
	Normal (2-56ng/ml)	1	2,9
	Total	35	100
Ferritin	High (>200ng/ml)	25	71,4
	Total	25	71,4
Insulin	Low (<10 µU/mL)	31	88,6
	Normal (10-100µU/mL)	4	11,4
	Total	35	100
HbA1c	Low (<80mg/dL)	30	85,7
	Normal (80-130mg/dL)	4	11,4
	High (>130mg/dL)	1	2,9
	Total	35	100

Table 2. The value of Hepcidin, Ferritin, Insulin, and HbA1c

No.	Levels	N	Min	Max	Median	Normal Range
1.	Hepcidin (ng/mL)	35	0.12	2.55	0.19	2-56
2.	Ferritin (ng/mL)	25	1059	9747	3283	20-200
3.	Insulin (µU/mL)	35	1.19	60.83	2.19	10-100
4.	HbA1c (mg/dL)	35	16.37	159.39	26.95	80-130

Table 3. Correlation between markers of iron overload with insulin levels and HbA1c levels in patients with β thalassemia

No.	Levels	N	Min	Max	Median	Normal Range
1.	Hepcidin (ng/mL)	35	0.12	2.55	0.19	2-56
2.	Ferritin (ng/mL)	25	1059	9747	3283	20-200
3.	Insulin (µU/mL)	35	1.19	60.83	2.19	10-100
4.	HbA1c (mg/dL)	35	16.37	159.39	26.95	80-130

The Pearson correlation test (Table 3) showed that hepcidin levels had no significant relationship with ferritin levels ($p = 0.964$; $r = -0.010$), but had a significant relationship with insulin and HbA1c levels ($p = 0.001$) in patients with β thalassemia. Hepcidin levels had a strong positive correlation with insulin ($r = 0.771$) and a very strong positive correlation with HbA1c ($r = 0.849$). Ferritin levels did not show a significant relationship with insulin ($p = 0.785$; $r = 0.057$) and HbA1c ($p = 0.420$; $r = 0.169$) in patients with β thalassemia.

4 DISCUSSIONS

The study revealed that most of the subjects were adolescents and children, but we also found two adult and one elderly thalassemia patient. Previous studies have shown that the quality and duration of life of β thalassemia patients had improved over the past ten years due to regular blood transfusions balanced with iron chelation therapy and better patient compliance (Mokhtar et al., 2013). Based on gender, the female was 51.4% of thalassemia patients, and 48.6% were male. The number of female patients is not much different from male patients. It is related that thalassemia is a genetic disease caused by a single autosomal recessive allele factor, not a congenital disorder caused by allele factors linked to sex chromosomes (Taher, Weatherall, and Cappellini, 2018).

Hepcidin levels in this study had a median value of 0.19ng / mL, and most of the subjects had a decrease in hepcidin levels. The reductions in hepcidin levels previously have been reported by several studies. Ineffective erythropoietic activity is the cause of the decrease in hepcidin synthesis in thalassemia patients (Huang et al., 2019; Jones et al., 2015; Pasricha et al., 2013).

Decreasing hepcidin levels will increase ferritin levels. The increased ferritin levels that reach the threshold will lead to the formation of free iron, which is toxic and can damage various organs, including the pancreas, which results in decreasing insulin secretion capacity and disruption of glucose metabolism to diabetes in thalassemia patients (Leecharoenkiat et al., 2016; Wang et al., 2014). This study showed decreased hepcidin levels below average in most subjects and increased ferritin levels in all study subjects. Still, statistically, there was no relationship between hepcidin levels and ferritin levels in the study subjects.

Ferritin levels in this study also did not show a relationship to insulin and HbA1c levels. It can be due to the measurement of ferritin data taken from patient medical records and not direct measurements that coincide with hepcidin, insulin, and HbA1c levels (Wang et al., 2014). Besides, serum ferritin levels can be influenced by various factors such as transfusions, iron chelation therapy, inflammatory disease, liver disease, and malignancy. The serum ferritin level is not the best examination to mark iron accumulation in various organs (Fernández-Real and Manco, 2014). Magnetic Resonance Imaging (MRI) and non-transferrin bound iron (NTBI) examinations have been reported to be better at assessing organ damage from iron accumulation (Elalfy et al., 2015).

This study showed a significant positive correlation between hepcidin levels and insulin levels in thalassemia patients; the lower the hepcidin level, the lower the insulin level. This result was the same as Al-Hakeim et al.'s result, which showed a positive correlation between hepcidin levels and insulin levels in patients with β thalassemia (Al-Hakeim, Al-Khakani, and Al-Kindi, 2015). Hepcidin is the main regulatory protein in regulating iron levels in the

body. The decrease in hepcidin levels in thalassemia patients contributes to the occurrence of iron overload conditions, which can cause pancreatic damage characterised by failure and decreased insulin secretion by pancreatic β cells (Huang et al., 2019).

Ineffective erythropoietic activity may cause a decrease in hepcidin synthesis. The increased erythroid expansion in the bone marrow will trigger the production of several erythroid factors such as erythrofferone, twisted-gastrulation 1 (TWSG1), and GDF-15, which directly reduces the hepatic synthesis of hepcidin (Tanno et al., 2009; Kautz et al., 2015). The decrease in hepcidin levels results in an increase in iron absorption in enterocyte cells. The rise of ferroportin activity and the mobilisation of iron stores from macrophages contribute to iron overload in thalassemia patients (Larissi et al., 2019).

Iron overload caused by decreasing hepcidin levels can cause an increase in free iron levels (NTBI) circulating in plasma and deposited in cells through the L-type voltage-dependent Ca2 + (LVDCC) and Zip14 channels (Leecharoenkiat et al., 2016). An increase in free iron levels beyond cells' ability to form ferritin can turn this free iron into toxic. This free iron through the Fenton reaction can produce ROS free radicals such as hydroxyl radicals (OH-) and hydroxyl anions (OH*), which can oxidise lipids, protein denaturation, organelle damage, and even cell death (Chutvanichkul et al., 2018). The pancreas is very susceptible to ROS because it has low antioxidants levels (Shams et al., 2010). Increasing ROS such as hydroxyl radicals in the pancreas can trigger apoptosis of pancreatic β cells through interactions between mitochondria and the endoplasmic reticulum. It also inhibits adenosine triphosphate production (ATP), failing and decreasing insulin secretion (Backe et al., 2016).

This study also showed a significant positive correlation between hepcidin levels and HbA1c levels; the lower the hepcidin level, the lower the HbA1c level. The reduction of hepcidin levels and HbA1c levels is still unclear. It might relate to hemoglobinopathy's coincidence in thalassemia patients (Zhang, Xiao, and Fan, 2018; Tsilingiris et al., 2019).

The hemoglobinopathy incidence among thalassemia patients may lead to abnormalities in the erythrocyte cell membrane leading to cell damage. The erythrocyte cell's destruction is faster, and the erythrocyte age is shorter than average (Hoffman et al., 2013). It can lead to reduced glycosylation time resulting in a decrease in HbA1c levels to below. On the other hand, hemoglobinopathy in thalassemia patients can cause chronic hypoxia due to anaemia. This hypoxic condition will increase erythropoietic activity, suppressing hepcidin levels' synthesis to be low (Huang et al., 2019).

Ji and colleagues assumed that a low HbA1c value in thalassemia patients could be misinterpreted compared to the standard reference. Other parameters than HbA1c, such as glucose levels and glycated albumin levels, are needed to appropriately assess glucose abnormalities in thalassemia patients (Ji et al., 2015; Wu et al., 2016).

5 CONCLUSIONS

The study found the lower the hepcidin level, the lower the insulin and hba1c levels. ferritin levels do not have a significant relationship with insulin and hba1c levels

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