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Investigating the level of Hba1c and insulin level in β-thalassemia patients

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ABSTRACT

Background: β-thalassemia is an inherited blood disorder characterized by reduced or no synthesis of β globin chain, resulting in chronic anemia, so blood transfusion is required as curative therapy. Repeated blood transfusions lead to iron overload that can lead to multiple organ damage, including pancreatic organs.

Objective: This study aimed to describe HbA1c and insulin levels of β-thalassemia patients. The study also tested whether there was a significant difference in insulin and HbA1c levels among patients with different β-thalassemia categories.

Method: This research was an analytic observational study. The samples were taken by total sampling and involved 30 patients, and the examination was carried out using the patient's blood plasma.

Result: The study found that samples had low HbA1c levels. There was no significant mean difference (p>0.05) between insulin and HbA1c in the mild, moderate, and severe clinical degree groups. There was no significant difference in average (p>0.05) insulin and HbA1c in the thalassemia sufferers with allele β+ and β0 types.

Conclusion: There was a decrease in insulin and HbA1c in patients with β-thalassemia, which may be caused by damage pancreatic organ damage.

INTRODUCTION

β- thalassemia is one of the world's most common autosomal recessive disorders and spreads in every country, with the highest incidence in countries called the Thalassemia Belt. As one country in the thalassemia belt, Indonesia has a prevalence of thalassemia carriers, reaching around 3.8% of the entire population. Based on data obtained from the Indonesian Thalassemia Foundation, there has been a continuous increase in thalassemia cases each year. Recently, the data indicated that the worst thalassemia cases in Indonesia occurred in 2022, with a reached figure of 10,555.[1] The frequency of beta-thalassemia genes in Indonesia ranges from 3 to 10%, and in Central Java, the prevalence of thalassemia is 0.02%.[2] A study conducted in Banyumas found a spectrum of beta-thalassemia mutation was about 8%.[3]

β-thalassemia is a hereditary blood disorder characterized by deficiency or absence of β globin chain synthesis. Deficiency of the β-globin chain causes reduced hemoglobin levels in erythrocytes and decreased erythropoiesis, resulting in anemia. Clinical signs of β-thalassemia include microcytic hypochromic anemia, the presence of nucleated erythrocytes on the peripheral blood image, and decreased or absent levels of hemoglobin A (HbA). β-thalassemia patients with severe anemia undergo routine blood transfusions as curative therapy, causing of the accumulation of iron.[4]

Beta-thalassemia patients are at risk of developing diabetes mellitus due to pancreatic hormone disturbance.[5] However, the HbA1C marker used in assessing diabetes control quality is not sufficient in diabetes patients with thalassemia.[6] There is a paucity of studies regarding the effect of β-thalassemia trait on HbA1c in non-diabetics. It is hypothesized that the β-thalassemia trait can falsely lower HbA1c through multiple mechanisms, such as ineffective

erythropoiesis and peripheral hemolysis, which can shorten the lifespan of erythrocytes, resulting in lower HbA1c.

Elevated hemoglobin F levels can also affect some laboratory methods used to measure HbA1c.[7] However, a cross-sectional study found that non-diabetic individuals with heterozygous β-thalassemia presented a similar mean HbA1c value to those without the thalassemia trait.[8] Another study reported a discordantly low HbA1c with an abnormal chromatogram caused by rare β-thalassemia. Clinically silent β-thalassemia may lead to low HbA1c values and abnormal chromatograms by HPLC. Therefore, differential diagnosis is essential, and further tests, such as GA, OGTT, hemoglobin electrophoresis, and genetic tests, are needed for differential diagnosis.[9]

The current literature needs to include more information on the type of mutation and the extent of pancreas tissue damage in beta-thalassemia patients. Therefore, this study aims to investigate the level of HbA1c and insulin levels in β-thalassemia patients and determine whether there is a relationship between HbA1C and insulin levels with beta-thalassemia mutation alleles.

METHOD

Study Design

This is a cross-sectional study design.

Setting and Respondent

Thirty β-thalassemia patients recorded in the Indonesian Thalassemia Foundation database were included in the study. The inclusion criteria were patients registered with mutant types in the lab database. The exclusion criteria for the gene mutation were unreadable. The data included the sample's age, sex, transfusion duration, clinical severity, and mutant-type alleles.

The Variable, Instrumen, and Measurement

The variables examined in this study were HbA1c level, insulin level, and mutation type. Venous blood samples of 3 mL were collected into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The samples were subsequently stored at 4-8°C to ensure stability.[10] Further analysis of the blood samples was performed using enzyme-linked immunosorbent assay (ELISA) to determine HbA1c and insulin values like the previous study.[11] Data HbA1c were reported in units of % and insulin in μIU/mL. The experiments were conducted at the research laboratory facilities of the Faculty of Medicine, Universitas Jenderal Soedirman.

Data Analysis

ELISA data were analyzed using GraphPad Prism version 12 to obtain HbA1c and insulin values. Univariate analysis was utilized to describe each variable. Tests of difference in Insulin and HbA1c levels among clinical severity, the difference of insulin, and HbA1c levels between type alleles were tested using bivariate analysis Kruskal-Wallis test and Mann Whitney Test. The insulin and HbA1c samples compare the standard values.

Ethical Consideration

A study has been approved by The Medical Ethics Committee of Universitas Jenderal Soedirman under registry number 150 / KEPK / VI / 2020.

RESULTS

Table 1 outlines the key characteristics of the 30 patients included in the study. The average age was 12.52±1.37 years. There was a slightly higher proportion of males than females. Patients had received an average of 7.52±0.84 years of regular blood transfusions. Most patients demonstrated a moderate clinical form of the disease, and more patients had the severe β0 allele while had the milder β+ form.

Table 1. Characteristics of thalassemia patients (n=30)

Characteristics	Result
Age, yrs	12.52 ± 1.37
Sex, M/F	17 (56.7%)/13 (43.3%)
Transfusion duration, yrs	7.52 ± 0.84
Clinical severity	
Mild	3 (10%)
Mediate	21 (70%)
Severe	6 (20%)
Type allel	
β+	12 (40%)
β0	18 (60%)

Analysis of insulin levels in the patient's blood indicated that 30 samples had low insulin levels (100%), with a minimum value of 1.226 μIU/mL and a maximum value of 41.86 μIU/mL. The mean score was 21.9 μIU/mL. Meanwhile, the levels of HbA1c in the sample of this study demonstrated that 30 samples had low HbA1c levels (100%), with a minimum HbA1c score of 2.2%, a maximum value of 4.3% and a mean score of 2.6±1.26% (Table 2). Table 2 suggested that the HbA1c levels tended to lower from the normal range (<5.7%), while the insulin levels were notably lower than average.

Table 2. The Difference on Insulin, HbA1c, Between Sample and Normal Value

Biomarker	Mean±SD	Normal Value
Insulin (μIU/mL)	21.9±0.86	10-100
HbA1c (%)	2.6±1.26	In-rank: <5.7; Prediabetes: 5.7-6.4; Diabetes: >6.4%

Table 3. Test of Difference on Insulin and HbA1c Levels Among Clinical Severity and Type of Alleles

	Clinical severity			p-value*	Type alleles		p-value**
	Mild	Moderate	Severe		β⁺	β⁰	
Insulin (µIU/mL)	2.97±1.23	6.07±1.14	12.00 ± 2.394	0.861	9.04±1.461	5.54±1.383	0.15
HbA1c (%)	2.53±1.72	2.67±1.45	3.12±1.73	0.562	2.72±1.14	2.81±1.19	0.062

*Kruskall Wallis Test; **Mann Whitney Tets

The results of tests for differences in insulin and HbA1c levels between groups are presented in Table 3. No significant differences were found in mean insulin or HbA1c values ($p > 0.05$) between thalassemia patients with mild, moderate, and severe clinical phenotypes. Additionally, no significant differences were detected in mean insulin or HbA1c levels ($p > 0.05$) between thalassemia patients with the β+ allele versus the β0 allele.

DISCUSSION

The results of the examination of sample insulin in this study showed a tendency for low insulin levels to be below-average values (Table 2). A previous study on 40 thalassemia patients in Bangkok showed a decrease in insulin levels below average in 52.5% of subjects.[12] The following factors may contribute to the low insulin levels in thalassemia patients. Iron overload is a common complication of thalassemia, and it can damage various organs, including the pancreas.[13] The pancreas is responsible for producing insulin, and damage to this organ can lead to insulin deficiency.

Another factor is impaired Glucose Tolerance. It is a condition in which the body cannot use glucose effectively, leading to high blood sugar levels. Thalassemia patients are at a higher risk of developing impaired glucose tolerance, which can contribute to insulin resistance and low insulin levels.[14] The condition also may lead to insulin resistance. The condition contributes to the body's cells becoming less responsive to insulin, leading to high blood sugar levels. Thalassemia patients are also at a higher risk of developing insulin resistance, which can contribute to low insulin levels.

The low insulin level in this study can also be explained through the molecular pathways of abundant iron cell deposits, which lead to the components of over-free radicals. The excessive production of free radicals, including superoxide anions and hydrogen peroxide, damages cellular components such as lipids, proteins, and DNA.[15] This oxidative damage disrupts the normal functioning of pancreatic cells, impairs insulin secretion, and contributes to pancreatic inflammation and fibrosis. Iron accumulation in beta cells impairs their ability to produce and release insulin in response to glucose. This dysfunction leads to inadequate insulin secretion and contributes to glucose metabolism abnormalities, including impaired glucose tolerance and diabetes mellitus. The excess iron is distributed to the pancreas, causing the formation of hydroxyl radicals through the Fenton reaction. The increase in hydroxyl radicals also induces apoptosis of pancreatic β cells, inhibits mitochondrial function, and failure of insulin secretion, resulting in decreased insulin levels.[16] In some cases of thalassemia, fibrosis (scarring) may occur in the pancreas due to iron deposition and inflammation.

The fibrotic changes can disrupt the pancreas's typical architecture and affect the beta cells' function, leading to reduced insulin production.[17] In action, pancreatic myofibroblasts hold significant sway over the intricate web of extracellular matrix deposition and the ensuing fibrotic response triggered by sustained signaling, consequently unraveling the molecular intricacies that underlie PMF activation and valuable insights into the biology of chronic pancreatitis. Molecules responsible for these actions include transforming growth factor-β1, platelet-derived growth factor, and an array of other microenvironmental cues, such as hypoxia and the unyielding stiffness of the extracellular matrix.[18] It is important to note that the relationship between thalassemia and insulin levels is complex, and more research is needed to understand the mechanisms involved fully. However, the results of this study suggest that thalassemia patients may be at a higher risk of developing insulin deficiency and resistance, which can contribute to low insulin levels.

The measurement of HbA1c levels in the subjects showed results below average values (2.6±1.26%) (Table 2). Research findings indicate that anemia in carriers of β-thalassemia without diabetes mellitus does not significantly impact HbA1c levels. This study suggests that individuals with β-thalassemia do not experience significant alterations in their HbA1c levels despite anemia. Several reasons account for this phenomenon: Thalassemia patients have a shorter lifespan of erythrocytes due to ineffective erythropoiesis and peripheral hemolysis, resulting in lower HbA1c levels.[19] Thalassemia patients may also have elevated levels of hemoglobin F, which can affect some laboratory methods used to measure HbA1c.[20]

Abnormalities in hemoglobin can theoretically produce false effects on HbA1c levels since HbA1c is measured against normal hemoglobin. In some cases, thalassemia patients may have clinically silent β-thalassemia, which can lead to low HbA1c values and abnormal chromato-

grams by HPLC.[21] Another reason is that thalassemia patients also receive fresh blood through transfusions every 2-4 weeks, in contrast to normal erythrocytes, which have a lifespan of 120 days or 12 weeks. However, it is essential to note that the correlation between thalassemia and lower HbA1c levels is of negligible clinical significance.[9]

Two subjects showed normal insulin levels compared to all subjects. These subjects corresponded with the β+ allele type that routinely performed regular transfusions above 9 mg/dL. Maintaining hemoglobin above 10 mg/dL can reduce the burden of organ damage because the body can adapt to good oxygen. Thalassemia patients have reduced hemoglobin production, leading to lower oxygen-carrying capacity. By maintaining hemoglobin levels above 10g/dL, thalassemia patients can ensure an adequate oxygen supply to their tissues, vital for overall health and well-being.[22] Thalassemia patients commonly experience symptoms related to anemia, such as fatigue, weakness, and shortness of breath.

Keeping hemoglobin levels above 10g/dL can reduce the severity of these symptoms, improving the patient's quality of life and daily functioning. It also can help stimulate the production of red blood cells, compensating for the inadequate production caused by the thalassemia mutation. By maintaining hemoglobin above 10g/dL, thalassemia patients can help support the proper functioning of these organs and reduce the risk of complications associated with low oxygen delivery.[23] It is important to note that the target hemoglobin level may vary depending on the specific circumstances and the patient's overall health. The patient's healthcare provider should determine the optimal hemoglobin target, considering factors such as the type and severity of thalassemia, individual symptoms, and other medical considerations.[24]

The two-subject mutation is mild, the β+ type, which does not eliminate the globin chain. There are still globin chains produced to account for the manufacture of adult hemoglobin. As a result, individuals with a mild beta+ mutation typically have less severe symptoms than those with more severe mutations.[25] The reduced production of beta-globin chains in mild beta+ thalassemia leads to an imbalance in the production of alpha and beta globin chains, resulting in excess alpha globin chains. This excess of alpha chains can form unstable aggregates and cause damage to red blood cells, leading to hemolysis (premature destruction of red blood cells) and anemia. However, the clinical severity is milder compared to individuals with severe mutations where little to no functional beta globin chains are produced. It is important to note that the clinical severity of thalassemia can vary widely, even among individuals with the same mutation.

Other genetic and environmental factors, such as co-inheritance of other hemoglobinopathies or variations in the production of fetal hemoglobin, can also influence the clinical presentation and severity of thalassemia.[26]

Test of differences in this study confirmed no significant difference in the mean levels of insulin and HbA1c among thalassemia groups- the mild, moderate, and severe clinical symptoms (Table 3). However, the average HbA1c and insulin levels appear slightly higher in the more severe clinical type than in the mild one. Similar findings were also confirmed for the group with β+ and β0 alleles in patients with β-thalassemia. Previous research has shown that heterozygous β-thalassemia has a small but significant effect on HbA1c levels in individuals without diabetes mellitus. The impact of β-thalassemia trait-associated anemia on HbA1c is negligible, however.

The findings support using HbA1c as a diagnostic criterion for diabetes mellitus in thalassemia populations.[8] However, a study showed that participants with iron deficiency anemia had significantly higher HbA1c levels than those with other types of anemia; the difference was more significant in participants with more severe iron deficiency anemia. The study also found that participants with sickle cell anemia had slightly higher HbA1c levels than participants with other types of anemia. However, the difference in HbA1c levels was not statistically significant. Iron deficiency anemia is the most likely type of anemia to affect HbA1c levels in non-diabetics. The study's findings suggest that HbA1c levels may not be a reliable indicator of blood sugar control in people with anemia. Including thalassemia.[7]

Compared to the data in this study, HbA1c is far below the normal threshold compared to the HbA1c values from previous studies. This can be caused by many processes, including factors such as age: HbA1c levels tend to be lower in children and adolescents with thalassemia than in adults. This is likely since children have more active bone marrow and can produce more red blood cells. Another reason is the severity level of thalassemia. People with more severe forms of thalassemia are likelier to have lower HbA1c levels. This is because they are more likely to be anemic and have higher fetal hemoglobin levels (HbF). HbF is a type of hemoglobin produced in the fetus and early childhood. It has a higher affinity for oxygen than adult hemoglobin, which can help to improve oxygen delivery to the tissues.

Treatment also influences the result; people with thalassemia who are treated with blood transfusions or chelation therapy are more likely to have lower HbA1c levels. This is because blood transfusions can help improve anemia, and chelation therapy can help remove excess iron from the body. People with thalassemia who also have other medical conditions, such as diabetes or chronic kidney disease, are more likely to have higher HbA1c levels. These conditions can interfere with the body's control of blood

sugar levels. It is important to note that HbA1c levels can fluctuate over time in people with thalassemia.[5,27,28]

Previous data stated that genetic abnormalities in the β globin gene caused clinical heterogeneity in β-thalassemia patients.[29] More than 300 mutation points are identified in the β globin gene. The genotype variability in the β globin gene is often insufficient to explain the difference in phenotype even with the same genotype.[23] The clinical phenotype of β thalassemia patients varies depending on the need for blood transfusion. The phenotype is associated with imbalance and excess of α and β globin chains. This clinical spectrum involves many factors, including types of mutations in the β gene as primary modifiers, secondary modifiers that lead to an improvement in the balance ratio of α and β globin chains, and tertiary modifiers, both genetic and non-genetic which improve or worsen the appearance and complications of the disease.[25] This fact may be why, in this study, the levels of HbA1C and insulin in beta 0 and beta + types are not significantly different.

CONCLUSIONS AND RECOMMENDATION

The findings of this research demonstrate that individuals with β-thalassemia exhibit diminished levels of insulin and HbA1c. Furthermore, the study revealed no significant differences in HbA1C and insulin levels between beta 0 and beta + types. In light of these findings, the study recommends further research to comprehensively understand the mechanisms underlying the observed low levels of insulin and HbA1c in β-thalassemia patients. Healthcare providers should be cognizant of the unique metabolic profiles of β-thalassemia patients, particularly the reduced insulin and HbA1c levels. Customized and vigilant monitoring of blood sugar levels and tailored insulin management are essential to ensure optimal glycaemic control and overall well-being for individuals with β-thalassemia.

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