Serum Preptin Level in Iraqi Beta Major Thalassemic Patients

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Abstract

Background Beta thalassemia syndromes are a set of hereditary blood disorders marked by a deficiency of beta-globin chain synthesis, result in decrease hemoglobin in red blood cells, anemia, and a reduced RBC production. Iron overload is a common finding in chronically transfused beta thalassemia major patients with possible effect on beta cell function and secretion. This study aimed to assess preptin level in the serum in beta major thalassemic patients, in order to indicate the effect of oxidative stress on preptin secretion. And explain preptin effect on bone cells. Subject and methods; A case-control study that was performed in the Ibn Albaladi Hospital (during the period from 1st of September 2020 to the end of January 2021. It included 48 beta major thalassemic patients and 36 subject as healthy control. Information was taken from each subject including age, diseases. Subjects with any cardiovascular diseases, hyperemesis gravidram, liver diseases, kidney diseases, bone disease, diabetes mellitus, and patients take corticosteroid as well as patients in childhood were excluded in this study. The biomarkers studied were: fasting serum preptin, insulin were assessed. Serum preptin and insulin were measured by ELISA technique. Results; The mean values of (Preptin, Insulin) in patients group were less than control group. There was a moderate direct significant correlation $P<0.01$ between preptin and insulin. Conclusion; The mean value of serum preptin was less in thalassemic major group than control group. And direct correlated with insulin level which is also reduced in thalassemic patients.

Keywords: *Preptin, insulin, thalassemic patients*

Introduction

Thalassemia is one of the most common diseases in the world, which is causes the rapid destruction of erythrocytes, and in order to retain red blood cells, patients need to undergo daily blood transfusions. Regular blood transfusions, however, cause iron overload, which can lead to complications such as heart disease, diabetes, osteoporosis, and kidney disorders(1).

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Thalassemia is graded into two classes based on the two polypeptide chains: 1. Beta (β) –thalassemia. 2. Alpha (α) - thalassemia. The globin chain is affected or the abnormal hemoglobin involved in beta-thalassemia is beta-globin gen. While in alpha-thalassemia, the alpha-globin gene affects the globin chain. Beta thalassemia can be categorized into three categories clinically: A. Thalassemia major, B. Thalassemia intermedia, C. Thalassemia minor or trait. Thalassemia major means the patient has serious anemia and may require blood transfusions for the remainder of their lives. Thalassemia intermedia is a type of anemia that causes mild to moderate anemia and necessitates blood transfusions on occasion. Patients with thalassemia minor, typically do not

require blood transfusions and appear healthy[2].

Thalassemia minor usually goes unnoticed and has no symptoms. If it does, mild anemia is the most common symptom. Extreme hemolytic anemia is normal in thalassemia major. The symptoms of thalassemia major can consist of:

• Fatigue • Inability to thrive • Jaundice • Spleen and liver enlargement

• Abnormalities of the bones, especially those of the face and pale skin[3].

The key determinant of thalassemia patients' survival is daily blood transfusion. Normal blood transfusions, on the other hand, can lead to severe complications, which can increase thalassemia patients' morbidity and mortality. Most of these complications are: -Iron deficiency. -Infections transmitted via blood transfusions, especially hepatitis B virus (HBV) and hepatitis C virus (HCV). -Thrombotic occlusions of the cerebral, portal, retinal, and coronary circulations have been identified in patients with excess iron as a result of other factors such as arterial stiffness and endothelial dysfunction[4]. Multiple endocrine dysfunctions are common in TM patients, including: Hypogonadism, Growth failure, Diabetes, Hypothyroidism, Hypoparathyroidism, less frequently, hypoadrenalism[5].

Patients with TM are seriously anemic and must undergo blood transfusions for the remainder of their lives. Iron builds up in different organs in patients due to frequent blood transfusions[6]. However, TM induces long-term extravascular hemolysis, which increases intestinal iron absorption while decreasing iron bioavailability. This phenomenon, when combined with several blood transfusions over time, could result in iron overload and an increase in the amount of iron ions in the body[7]. As a result, ROS production will be encouraged. Oxidative stress is caused by a change in the balance between ROS and the antioxidant protection mechanism, which promotes ROS[8]. Excess ROS react immediately with other molecules, causing many organelles, especially the membrane, to malfunction in the cell, resulting in cytotoxicity and organ failure[9]. In the visceral organs (primarily the heart, liver, and endocrine glands), iron deposition as well as oxidative stress cause tissue damage and, finally, organ dysfunction or failure[10].

Preptin, an oligopeptide secreted by pancreatic beta-cells, is involved in glycometabolism and bone metabolism. Preptin is a 34-amino-acid peptide hormone discovered in 2001 that is co-secreted with insulin and amylin from secretory granules derived from cultured murine beta-cells and corresponds to Asp69-Leu102 of the proinsulin-like growth factor II E-peptide[11]. Proteases cleave preptin at the 21st phenylalanine amino acid residue. The truncated preptin peptide (preptin 1–16) that results from this cleavage has no effect on insulin secretion. However, full-length (34-amino-acid) preptin enhances glucose-mediated insulin secretion physiologically[12]. Preptin increases osteoblast replication while decreasing apoptosis. Preptin administration increases bone area and mineralizing surface. As a consequence, preptin plays a role in bone anabolism and contributes to bone mass preservation in hyperinsulinemia disorders like obesity[13]. The (1-16) N-terminal fragment of preptin is responsible for its bone function. This peptide promotes osteogenesis by phosphorylating p42/ 44 mitogen-activated protein kinases via a G protein coupled receptor[14]. Preptin levels in the blood are reduced in osteoporosis and osteopenia patients, and they are attributed to bone mineral densities BMD. As a result, preptin plays a role in osteoporosis pathogenesis, most likely by bone development rather than bone resorption[13].

Osteopenia and osteoporosis are common causes of morbidity in thalassemia patients of both genders. The pathogenesis of osteoporosis in TM is complex, and it varies from the pathogenesis of nontransfused patients' bone deformities, which are triggered by inadequate haemopoiesis and progressive marrow expansion. The loss of bone mass in TM has been related to a variety of factors. Ineffective haemopoiesis

with progressive marrow expansion, direct iron toxic effects on osteoblasts, impaired sexual maturation, growth hormone (GH) and insulin growth factor (IGF)-1 deficiency, parathyroid gland dysfunction, diabetes, hypothyroidism, and also liver disease, have been identified as possible causes of thalassemia-induced osteoporosis[15].

Method

Specimens were collected during the period from 1st of September 2020 to the end of January 2021. A total of eighty four subjects included in this study were divided into two groups: The first group (patients) forty eight subjects have beta major thalassemia as a thalassemic group their age from 18 and above. The second group (healthy control) thirty six subjects (not thalassemic) as a control group their age (18 – 35 years). Thalassemia participants were selected from Ibn Albaladi Hospital.

Subjects with any cardiovascular diseases, hyper emesis gravidram, liver diseases, kidney diseases, bone disease, diabetes mellitus, and patients take corticosteroid as well as patients in childhood were excluded in this study.

Statistics

Continues data were Described as median and interquartile ranges, mean± standard error of the mean (SEM). Student's t- test was used to analyze and compare between the means of the markers and variables between the patients and control. Pearson correlation was performed to test significant correlation among the parameters. Alpha level for statistical significance was set top < 0.05. Statistical Microsoft excel software version 2019 and IBM SPSS Statistics 26.0 software (IBM SPSS Inc., Chicago, IL) were used for statistical analysis. Graph Pad prism version 8 was used for standard curve fitting.

Results

The mean values of (Preptin, Insulin) in patients group were less than control group.

Table (1) Descriptive statistics and comparison of means of the study parameters between thalassemic and control groups

	Statistic	n	Median (Interquartile range)	Mean± Standard error of the mean	P value t-test
Preptin pg/ml	Patients	48	61.13 (46.84-74.53)	69.86± 6.98	< 0.001
	Control	35	131.56 (83.89-164.4)	124.36± 8.52	
Insulin mU/L	Patients	48	3.19 (2.56-4.86)	4.06± 0.31	< 0.001
	Control	35	8.34 (4.97-9.83)	7.98± 0.71	

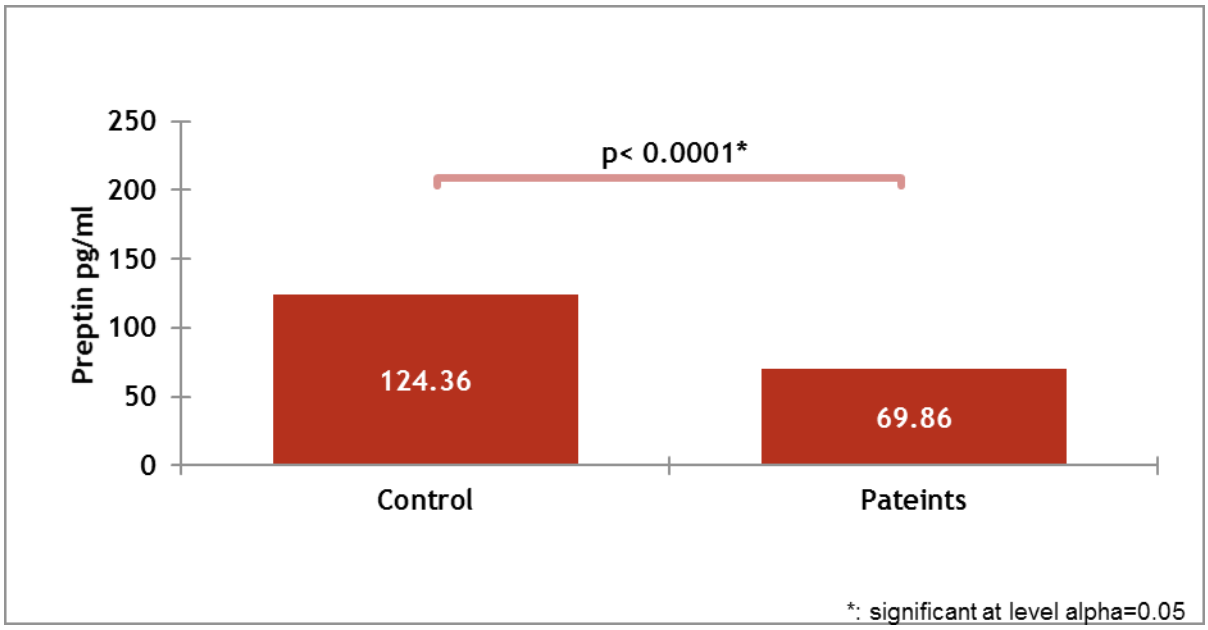

Figure 3-1 Bar chart compering means of serum preptin between patients and control groups using student's t-test. Significant P-value is set at alpha = 0.05

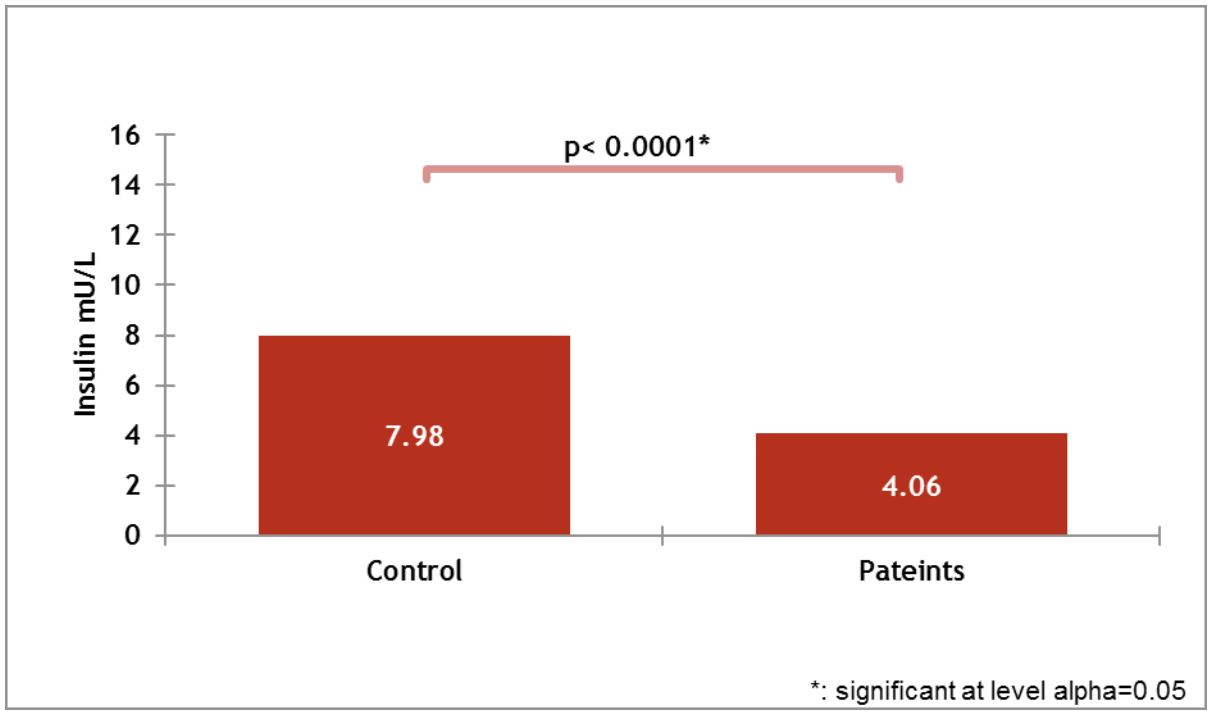

Figure 3-2 Bar chart compering means of serum Insulin between patients and control groups using student's t-test. Significant P-value is set at alpha = 0.05

Table 2 Pearson Correlation in the patients group. Presenting the correlation coefficients and p-values for serum Preptin and insulin.

		Preptin	Insulin
Preptin	r		0.36
	p		0.01
Insulin	r	0.36	
	p	0.01	

There is a moderate direct significant correlation P<0.01 between preptin and insulin in patients group.

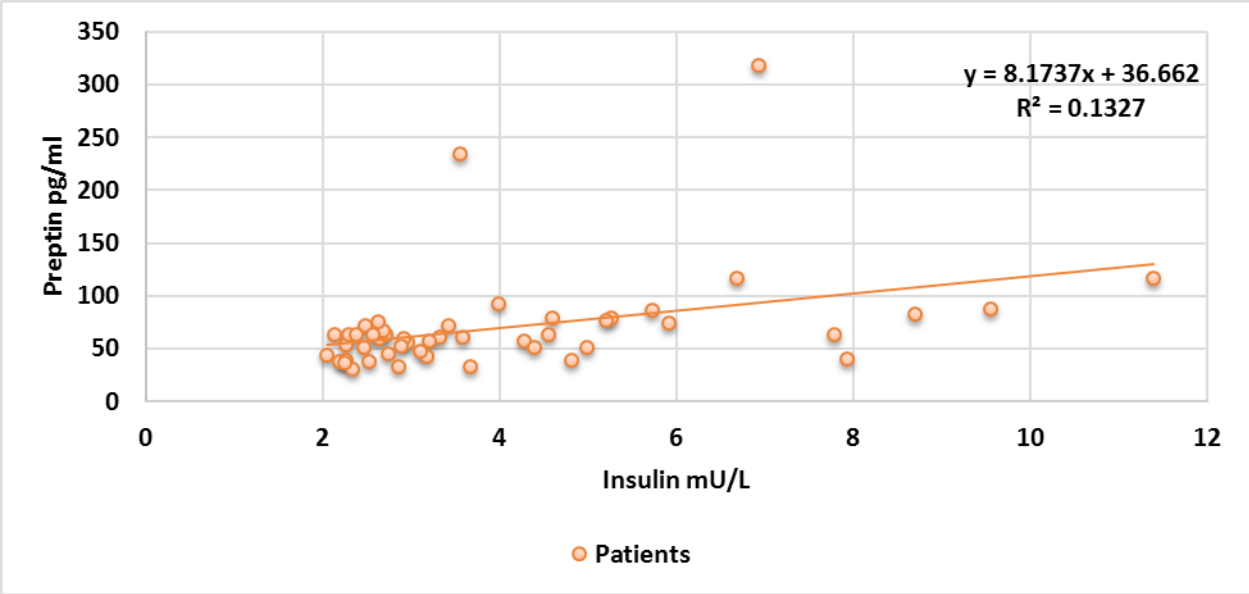

Figure 3-3 Scatter plot showing the correlation between (Preptin pg/ml & Insulin mU/L) in the patient's group, coefficient equation, model fit R2 and regression line plotted to ease the interpretation

Discussion

Beta thalassemias are heterogeneous autosomal recessive hereditary anemias characterized by reduced or absent β-globin chain synthesis [16]. There is damage and premature destruction of RBC precursors, causing ineffective erythropoiesis leading to severe anemia and compromised oxygen transport. In some patients, death would result without chronic blood transfusions. Laboratory abnormalities include microcytic anemia with abnormally shaped RBCs and abnormal Hb electrophoresis [17]. A Preptin hormone serum level was decreased significantly high in patients group than control group. But from other previous studies that concerned with serum ferritin, iron overload and oxidative stress in thalassemia major can explain that the increase of serum ferritin and iron overload occur from chronic blood transfusion result in oxygen reactive species generation and oxidative stress that affect pancreatic beta cells leading to secretion dysfunction [9, 10, 18]. Insulin serum level also showed a significant decrease in patients group than control group. This result agreed with previous study that represent insulin deficiency and insulin resistance in thalassemia major as a complication of disease in patients with hyper transfusion due to the high

serum ferritin and iron overload on different body tissue which cause pancreas and liver impairment as well as impact pancreatic islet cells [19]. Reactive oxygen species caused exclusively toxic effects and were associated with pathologies. Because of their high reactivity, ROS react with all types of biological molecules. Thus, high and sustained concentrations of ROS can cause damage to many cellular and extracellular constituents, including DNA, proteins, lipids, carbohydrates, and nucleic acids, often inducing irreversible functional alterations or even complete destruction [20]. As a result of these effect of oxidative stress, most of thalassemic patients may have functional decline in most of systemic glands especially pancreas. And these results have been proved by measuring some of pancreatic enzyme preptin and insulin which had indicated beta cells status [11,21]. Furthermore, there was also direct significant correlation relationship between preptin and insulin observed in correlation (Table 1 and 2). This agreed with other previous study that explained the secretion of preptin peptide from beta cell. Preptin was co-secreted with insulin and also sometimes enhance insulin secretion. So this result was indicator of beta cell status and represented the beta cell dysfunction in thalassemic group [12,22].

Conclusion

The mean value of serum preptin was less in thalassemic major group than control group. And direct correlated with insulin level which is also reduced in thalassemic patients. However, preptin also indirectly correlated with ferritin level but it was not significant.

Recommendation

1. Further studies about thalassemia major patients and osteoporosis needed that represent more clearly mechanisms that cause osteoporosis.

2. Expanding this study for explain the mechanism of preptin hormone on bone cells in thalassemic patients with osteoporosis in different age and gender using DEXA, vitamin D3, parathyroid hormone and other clinical measurements determine the degree of osteoporosis.

3. More studies about preptin hormone and it's correlation with insulin in thalassemic patients with diabetes mellitus type 1.

4. Genetic studies involve B-thalassemia and B-cell destruction could be involved in upcoming studies.

Conflict of Interest: None

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Ethical Clearance: Not required

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The Effectiveness of Potassium Iodide (KI) on Hard Dental Tissues Receiving Silver Diamine Fluoride (SDF) in Occlusal Surface: An in Vitro Study.