Assessment of Preptin as Predict Marker for Complication in Thalassemia Major

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ABSTRAC

Hemoglobin is made up of chains of protein called globin chains, which are broken down by genetic abnormalities that cause anemia. In many parts of the world, thalassemia is a serious public health problem [1]. It's also known as Mediterranean anemia. This condition is accompanied by several biochemical alterations in the blood. Some biochemical indicators of thalassemia patients were examined and compared to a healthy control group in this study. Serum iron, ferritin, TIBC, transferrin, transferrin saturation percentage, Preptin, insulin, and lipid profile are among these markers. Preptin, iron, lipid profile, blood sugar level, and insulin resistance in the serum of patients with thalassemia were the primary objectives of this study, as well as the association between cytokine(preptin), lipid profile, blood sugar level (and insulin resistance). Material and Methods: Fifty-eight Iraqi thalassemia major patients were participated in the current investigation. They were between the ages of 1 and 28. During the months of August 2018–January 2019, these patients were registered as thalassemic patients at the "Thallassemia Unit" at the "AL-Zahra'a Teaching Hospital" in Najaf, Iraq. Thirty otherwise healthy individuals were used as a control group. Their ages corresponded to those of the patients. None of these participants were anemic or suffered from any clear systemic or chronic disorders. The glucose levels, lipid profile, TIBC, and IRON concentrations were determined using a colorimetric technique. The levels of ferritin, insulin, preptin, salusin *α*, and salusin β were evaluated and quantified using the ELISA method. Special formulae were used to determine the Body Mass Index and Homeostatic model assessment of insulin resistance (HOMA-IR) and HOMAβ. Results: The investigation observed a substantial rise in blood iron, ferritin, TS percent, FBG, Insulin, TG, HOMA-IR, and preptin in individuals with Thalassemia Major when compared to the healthy group, but a considerably lower level in BMI, UIBC, Total cholesterol, HDL-C, LDL-C, and HOMA- β, (p›0.001). The impacts of age, TIBC, Transferrin, and VLDL-C were not significant. Conclusions: The current study found that serum preptin, a newly bioactive peptide with potent hemodynamic activities, can be used to detect the onset and progression of heart disease and renal disease, and may be a novel therapeutic candidate for the treatment of Major Thalassemia patients, particularly the cardiovascular system. Insulin resistance has a function in the progression of diabetes in people with thalassaemia.

Keywords: Major thalassemia, Preptin, HOMA-IR.

1. Introduction

Thalassemia comes from a mixture of two Greek words: thalassa denoting the sea [2, 3] that is the Mediterranean and anemia, weak blood. Cooley's anemia, which is another term often found in literature, was supposed to be endemic.

Beta Thalassemia is a hereditary anemia that results from errors in the development of beta-globin chains. Reduction in the production of hemoglobin beta chains and hemoglobin beta chains are the main characteristics. Anemia and high RBC turnover are common in beta-thalassemia due to poor hemoglobin synthesis; however, frequent blood transfusions can alleviate the symptoms of the disorder.

Thalassemia, once known as Cooley anemia, is caused by mutations in the globin gene loci on chromosomes 16 and 11, which alter the generation of globin protein [4-6].

TM patients almost want frequent blood transfusion (BT) for their being alive.

Hemolysis of red blood cells caused by poor hematopoiesis and frequent BT result in iron overload, as the human body lacks an active secretion mechanism for extra iron [7]. In the absence of treatment, it can result in anemia, splenomegaly, and severe bone deformations. By the age of twenty, the disease has progressed to the point of death. Blood transfusions are administered on an as-needed basis; splenectomy is performed if splenomegaly is evident; and therapy for iron excess induced by transfusions is administered. Tissue transplantation may be used to cure the disease [8].

Preptin is a peptide that was initially found in 2001 and is mostly synthesized by beta cells in response to postprandial glucose levels in the blood. It is responsible for physiological insulin production as a result of glucose concentration [9].

Preptin also contributes in regulating carbohydrate, lipid, and protein metabolism [10].

Preptin is largely generated in the pancreas, salivary glands, mammary glands, and kidneys [9, 11].

Preptin has an anabolic impact on bone that works in tandem with insulin hormone's activities. Preptin has also been shown to increase insulin secretion [9]. In *vitro* and in *vivo*, preptin promotes cell development and activity in osteoblasts and osteoclasts [12-14].

2. Materials and Methods

During the months of September and December 2018, a case-control research was carried out in the "Thallassemia Unit" of the "AL-Zahra'a Teaching Hospital" in Najaf, Iraq, at the "Thallassemia Unit." Patients with Iraqi thalassemia major were enrolled in this study, which comprised sixty of them. The participants' ages ranged from one to twenty-eight, while the control group consisted of thirty healthy persons. The volunteers' ages matched the patients'. This group had no visible systemic illnesses or chronic diseases, nor were they anemic. The Body Mass

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Index was calculated by dividing the individual's weight in kilograms by the length in square meters of the individual: BMI = (weight in kg) / (height in meters2) [15]. The serum was extracted from fasting venous blood samples from patients and control group participants and refrigerated at –20 0C until testing. TIBC was measured colorimetrically [16].

The ferritin levels were measured using an ELISA assay (Elabscience, USA). The transferrin saturation percentage was estimated employing the formula below: Transferrin (g/L) =Serum iron (µmol/L) / Transferrin (g/L) (3.98 X TS%) (Morgan, Dean, & Davies, 2002). Using a colorimetric technique, the fasting blood glucose and lipid profile (TC, TG, LDLC, and HDL-C) levels were estimated using a kit. Insulin levels were evaluated by the use of an ELISA test (CALBIOTCH Company,USA) In order to calculate the insulin resistance index (Homeostatic model assessment-insulin resistance, HOMA-IR), the steps below were taken: HOMA IR = [glucose (in mg/dl) * insulin (µU/ml)] / 405 , HOMA β %=360× insulin/(Glucose - 63) [17].

The presence of preptin was detected using an ELISA test (Elabscience, USA).

3. Statistical Analysis

The statistical analysis was done employing SPSS version 21 and Graphpad Prism version 5. (GraphPad Prism). In the case of continuous variables, the mean minus the standard deviation was used (SD). An equal frequency variable was tested using the Paired t-test and the independent t-test. Each variable's normalized Pearson coefficients were used to calculate bivariate correlations. It was determined that the *p* values obtained were less than 0.05 and 0.01 respectively, and that they were statistically and extremely statistically significant.

4. Results and Discussion

Table 1 compares the anthropometric and biochemical features of Thalassemia patients to a healthy control group. The current investigation demonstrated no statistically significant age difference between thalassemia patients and the control group. When comparing groups of thalassemia patients with healthy controls, the results of the BMI test indicated a statistically significant drop (p0.05) in BMI. When comparing patients with Major Thalassemia to the healthy group, researchers discovered a statistically significant increase in serum iron, ferritin, UIBC, TS%, FBG, Insulin, HOMA- β, TG, cholesterol, HDL-C, LDL-C, and preptin, but a statistically significant decrease in HOMA-IR (p›0.001), with the exception of TIBC, Transferrin, and VLDL-C being non-significant.

As shown in Table 2, Preptin and investigated factors in Major Thalassemia patients. HOMA-IR exhibits a strong positive correlation (P<0.05). BMI has a negative association.

Modeling Hemostasis HOMA [18] is a straightforward approach for evaluating insulin resistance and beta-cell activity using fasting plasma glucose and insulin measurements [19]. Patients with thalassemia major had lower HOMA- β and higher HOMA-IR levels than healthy controls [20] or lower HOMA- β and higher HOMA-IR levels over time [21]. HOMA- reduced in non-diabetic individuals, as in earlier research.

They also show a decreased β -cell function as evidenced by a significantly lower HOMA β – cell. It has been shown that the usage of frequent blood transfusions in TM patients has enhanced their quality of life while also increasing their overall survival.

On the other side, blood donations result in chronic iron overload, which frequently manifests as endocrine problems, most notably aberrant glucose metabolism such as diabetic mellitus (DM) and impaired glucose tolerance (IGT) [22].

Table (1): The anthropometric and biochemical characteristics of individuals with significant thalassemia and controls

P-value	Control (n=30) Mean±SD	Patients (n=58) Mean±SD	Parameter
N. S	17.97± 6.990	17.28± 5.758	Age (Yrs)
0.000**	29.11± 14.46	17.67± 3.73	BMI (kg/m2)
0.000**	123.70± 48.05	3472.22±2356.84	Ferritin(ng/ml)
0.000**	23.83± 6.096	35.68± 8.19	IRON (µmol/L)
0.175 NS	68.81± 12.61	63.56± 19.01	TIBC (µmol/L)
0.000**	44.98± 12.47	27.88± 15.03	UIBC
0.000**	35.52± 11.78	58.71± 13.34	TS%
0.175 NS	0.17± 0.03	0.16± 0.05	Transferrin(g/L)
0.000**	86.41±10.45	123.79±26.01	FBG (mg/dl)
0.000**	5.27±2.17	10.20± 4.79	Insulin (mIU/mL)
0.014*	1.84±1.34	2.82± 1.92	HOMA-IR
0.001**	89.86±13.24	66.74±10.48	HOMA-β%
0.000**	83.01± 8.73	86.01±6.24	TG (mg/dL)
0.000**	136.53± 20.09	122.07± 12.05	Cholesterol(mg/dL)
0.000**	47.47± 4.05	24.62± 2.43	HDL-C(mg/dL)
0.002**	91.91± 16.23	82.38± 11.76	LDL-C(mg/dL)
0.464 NS	17.62± 2.96	16.88± 5.08	VLDL.C
0.000**	125.25± 18.30	129.25± 20.58	Preptin (pg/ml)

BMI: BMI is an abbreviation for body mass index. FBG stands for fasting blood glucose. HOMA-IR is an abbreviation for hemostasis model assessment-insulin resistance. HOMA- β%: hemostasis model assessment-percentage of beta cells. The terms TG and HDL-C (high-density lipoprotein cholesterol) and LDL-C (low-density lipoprotein cholesterol) are interchangeable. The data is shown as Mean ±SD: standard deviation,

NS= nonsignificant distinctions at (P>0.05), and

NS= not significant distinctions.

*=statistically significant changes at (P≤ 0.05).
**=significant distinction at (P≤ 0.01
The high light spot of the current result has identified anovel peptide ,preptin as afirst clinical study for patients wih β-thalassemia major.
Preptin is recognized as a new peptide that cosecreted with insulin in order to enhance the amount of insulin secreted from pancreatic β-cells [23]. Preptin levels may be higher in individuals with TM due to increased or reduced secretion preptin metabolism. This needs to be clarified by more research. Yang et al. previously completed a research using T2DM human volunteers. Preptin levels were found to be much greater in DM patients than in normal persons [24]. Diabetes, on the other hand, is a common consequence of β -thalassemia major.

Table (2): Correlation between Preptin and examined parameters in patients with Major Thalassemia.

Parameters	r	P-Value
Age (Yrs)	-0.098	0.607
BMI (mg/dl)	-0.407	0.026*
FBG (mg/dl)	0.044	0.818
Insulin(µIU/ml)	0.158	0.410
HOMA-IR	0.458	0.011*
HOMA-β	-0.158	0.405
Ferritin (ng/ml)	0.065	0.733
IRON (µmol/L)	0.076	0.690
TIBC (µmol/L)	-0.033	0.862
UIBC (µmol/L)	-0.071	0.711
TS%	0.102	0.593
Transferrin (g/L)	0.033	0.863
HDL.C (mg/dl)	-0.103	0.590
VLDL.C(mg/dl)	-0.142	0.456
LDL.C(mg/dl)	-0.084	0.658
Cholesterol (mg/dl)	-0.116	0.541
TG (mg/dl)	0.162	0.391

P- Value 0.05 = significant, r : Pearson correlation. *=significant differences at (P≤ 0.05), **=significant differences at (P≤ 0.01).

Prevalence estimates vary widely, ranging from 1% to 21% of patients accessing a thalassemia service [25, 26]. In part, this heterogeneity might be attributed to the advent and widespread use of successful iron chelation treatment, but it can also be attributed to the ages of the individuals under investigation, with lower rates observed in younger patients. Many etiological variables may contribute to diabetes in thalassemia patients. Transfusional iron excess contributes to pancreatic beta-cell death and diabetes development.
Thalassemia patients who have poor chelation therapy compliance and who are older when they begin treatment are both at risk of having impaired glucose metabolism [27]. Other possible a etiological factors include altered β -cell insulin secretion, [28], autoimmunity, [29] insulin resistance secondary to liver disease and Hepatitis C virus infection affecting glucose metabolism [30].
Furthermore, because of the worldwide epidemic of diabetes, people with thalassemia may acquire type 1 or type 2 diabetes, regardless of whether or not they have thalassemia in the first place. In a recent research Aslan et al. [31], patients with GDM and glucose intolerance had higher blood preptin levels than those in the control group. Other investigations have shown that individuals with GDM have higher serum preptin levels.
According to Baykus et al. [32], a decreased preptin level was discovered in the GDM group (as in disagreement with the current study). It is possible that low preptin levels are responsible for this decline in ß cell activities. The quantity of insulin administered should have decreased as a result of these conditions. They believe, however, that no such decline in insulin levels was discovered as a result of the development of insulin resistance in pregnant women [9].
It was shown that elevated preptin levels in diabetic people were linked to increased insulin resistance (HOMA-IR) by Yang and colleagues, but that preptin levels and insulin secretion were not connected in any way. The current study and Buchanan's findings on insulin resistance are both compatible with this finding. There was speculation about a possible link between preptin and insulin secretion, however the researchers did not uncover a correlation between preptin and insulin secretion [24]. Previous studies have found a link between Thalassemia Major and a greater blood preptin level in newly diagnosed individuals; hence, it is possible that preptin is involved in its pathogenesis.

5. Conclusions

Preptin is a newly discovered bioactive peptide with significant hemodynamic activity, and measurement of serum Preptin levels may be a novel technique to identifying patients with serious Thalassemia. The present result show increase in serum preptin levels in newly results in patients with Major Thalassemia to provide a novel approach to identify the severity of iron overload complication with metabolic changes such as Insulin resistance .

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