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Short article

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Correlation of Klotho Protein with Troponin-I as a Marker of Myocardial Damage in Iraqi Beta-Thalassemia Major Patients

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Abstract

Background: Beta thalassemia is a hereditary blood condition characterized by a decrease or absence in the production of the beta-globin chain of hemoglobin. Patients with beta-thalassemia major often require regular blood transfusions and are at an increased risk of developing complications such as iron overload and cardiac injury. In recent years, there has been increasing interest in Klotho protein as a multifunctional protein known for its anti-aging and cardio-protective properties. Several studies have revealed a potential correlation between Troponin, a protein released into the circulation as a result of heart muscle damage, and the level of Klotho protein.

Methods: This study included thirty Beta-Thalassemia Major (β-TM) patients and thirty control healthy subjects. Levels of Klotho protein and Troponin-I were determined using the ELISA technique and measured for all participants.

Results: Serum Klotho protein and Troponin-I levels were significantly elevated in β-TM patients compared to healthy control subjects ($P< 0.001$). A positive correlation was found between serum Klotho protein and Troponin-I in the β-TM patients' group.

Conclusion: A positive correlation was found between serum Klotho protein and Troponin-I in the β-TM patients, which may highlight a relationship between Klotho and cardiac damage.

Keywords: Beta-Thalassemia Major, Cardiac dysfunction, Klotho protein, Troponin-I, Iraq.

Introduction

Beta-thalassemia major (β-TM) is an inherited blood disorder that causes abnormalities in the production of beta chains of hemoglobin. This leads to a range of phenotypes, from severe anemia to individuals who show no clinical symptoms (1). The subject matter was categorized into three different forms: major, intermediate, and minor (2).

Cardiovascular disease continues to be the primary cause of mortality in both Transfusion-dependent thalassemia (TDT) and Non-transfusion-dependent thalassemia (NTDT) patients, with a prevalence rate of 71%. Iron overload is the main cause of cardiac injury in these individuals. It was brought about by hemolysis, frequent blood transfusions, increased absorption of iron in the intestines, and a deficiency in the body's ability to excrete iron (3).

Klotho protein is a protein that may be found either attached to the cell membrane or in a soluble form. It has the ability to slow down the aging process and has properties that help prevent oxidative damage and cell death (4).

It impacts multiple metabolic pathways that are crucial for the development and prevention of cardiovascular disorders. Klotho inhibits the

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process of lipid peroxidation and reduces inflammation. Additionally, it protects against damage to the inner lining of blood vessels and the formation of calcium deposits. Klotho reduces the stiffness of blood vessels and inhibits the formation of fibrous tissue in the heart (5).

Troponin is an intracellular protein that plays an essential role in regulating striated muscle contraction (6). It is secreted into the bloodstream following a myocardial injury, and the level of troponin is directly correlated with the quantity of damaged cardiac myocytes (7). The objective of this study is to explore the underlying relationship between Klotho protein and troponin-I as a marker of myocardial damage in individuals with major beta-thalassemia in Iraq.

Materials and Methods

Study participants

This study was a cross-sectional design performed in the Ibn Albaladi Center of Blood Diseases in Baghdad during the period from the 1st of March 2023 to the end of August 2023. This study included sixty participants living in Baghdad (34 males and 26 females) ranging from 18-30 years of age, divided into thirty healthy subjects (17 males and 13 females) and thirty patients with Beta-Thalassemia Major on iron chelator therapy (17 males and 13 females). The Scientific Committee Biochemistry Department, College of Medicine, University of Baghdad, Iraq approved this study. Participants filled out questionnaires to get agreement for this study to collect information on Healthy subjects and patient groups.

Exclusion criteria

Patients with Diabetes mellitus, Brain and Kidney disease, Drugs such as metformin, statin (atorvastatin, pitavastatin, simvastatin fluvastatin), Renin-angiotensin-aldosterone inhibitors (losartan, valsartan), mTOR inhibitor (rapamycin, everolimus) and Metformin, Cancer, Obesity, Active Infection and participants with history of cardiovascular disease were excluded in this study.

Sample collection

Blood samples were collected from the subjects' veins from 8:00 a.m. to 11:00 a.m. and preserved using a disposable syringe with a capacity of 10 ml. The gel tubes containing blood were centrifuged in 2000 (Xg) for 10 to 15 minutes. The serum was divided and preserved at a temperature of -20 °C using sterilized Eppendorf tubes. 0.5ml of serum was used until the analysis of serums Klotho and Troponin-I was performed.

Biochemical analysis

Determination of Serum Klotho and Troponin-I by enzyme-linked immunosorbent assay (ELISA) kits. Klotho-(KL)-SEH757Hu Cloud-Clone Corp (USA), Troponin-I - SEA478Hu Cloud-Clone Corp (USA).

Statistical analysis

The Student's t-test has been used to examine and compare the means of the markers and variables between the patients and control group. A Pearson correlation analysis was conducted to determine if there was a significant association between the parameters. The alpha level for statistical significance was set to $p < 0.05$. Statistical analysis was measured using the program MedCalc version 19.6.1

Results

Demographic characteristics of the β-TM Group (n = 30) and control subjects (n =30) enrolled in the present study are shown in Table (1). There was no significant difference in the frequency distribution of individuals according to gender between beta thalassemia major and control group, with 17 (57.0 %) and 13 (43.0 %) males and females in each group. There is a significant difference in BMI across the groups with a p-value <0.01. The β-TM group had a Mean±SD (21.12± 0.95 Kg/m2), and the Control group had a Mean±SD (23.72± 1.41 Kg/m2) (Table 1).

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Table 1. Demographic and Laboratory data among β-Thalassemia Major (β-TM) and Control groups.

Parameter	Control (n = 30)	β-Thalassemia Major (β-TM) (n = 30)	P value (> 0.05)
Male	17	17	
Female	13	13	
Age (Years) (Mean± SD)	22.83± 2.03	22.67± 4.78	0.987 ns
Klotho level (ng/mL) (Mean± SD)	1.48± 0.51	5.34± 0.57	<0.001***
Troponin level (pg/mL) (Mean± SD)	436.67± 163.48	1472.10± 499.59	<0.001***

ns: non-significant, *** significant

The mean±SD values of Serum Klotho for β-TM and Control are 5.34±0.57 (ng/mL), and 1.48±0.51 (ng/mL) respectively. β-TM group exhibit significantly higher mean values than control group, with a p-value less than 0.001, indicating a statistically significant difference (Table 1).

For Troponin, the Mean±SD values for the β-TM group and Control group are Mean±SD (1472.10±499.59 pg/mL) and (436.67±163.48pg/Ml), respectively (Table 1).

There is a positive correlation between Troponin and klotho protein, this relationship suggests that individuals with higher troponin levels tend to have higher Klotho levels.

The positive correlation (0.45) could reflect a relationship where both markers increase in response to cardiac stress or dysfunction.

Discussion

The mean body mass index (BMI) in the present study showed a significant difference in β-TM patients compared to the control which is approximately similar to the findings of Harbi NS et al and Mettananda, S.et al. (8,9).

The harmful effects of iron chelator therapy, resulting in excessive accumulation in tissues and affecting iron-dependent enzymes involved in collagen modification, are believed to be responsible for growth abnormalities. Furthermore, it is believed that insufficient hemoglobin, elevated ferritin levels, and inadequate iron chelation are other contributing factors. Another study demonstrated similar findings, indicating that patients with thalassemia major exhibited a decreased growth rate and lower BMI. These effects were linked to low levels of hemoglobin, elevated ferritin levels, and inadequate iron chelation (10).

Thalassemia patients experience oxidative stress and inflammation due to the direct effects of iron poisoning. Su and Yang determined that α-Klotho may function as an acute-phase response mechanism, according to the observed elevation of serum α-Klotho protein levels in response to restraint stress. α-Klotho functions as an anti-inflammatory regulator by controlling the synthesis of nuclear factor-κB-related inflammatory proteins, leading to a decrease in the production of various pro-inflammatory cytokines and oxidative stress damage. α-Klotho provides cellular and defense against oxidative stress (11,12).

Prior studies have demonstrated an increased level of production of Klotho protein in the cardiomyocytes during ischaemia/reperfusion (I/R) in order to safeguard the ischemic heart from more damage, serving as a cardioprotective mechanism (13).

the Troponin level in this study had a significant difference in β-TM patients compared the Control group These results are consistent with Saeed's findings (14).

Troponin is a specific cardiac biomarker that indicates the initial phases of damage to the heart muscle. Following apoptosis and the cessation of cellular metabolism, troponin is

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released from meiotic cells, leading to an increase in its presence in the serum as a result of cellular injury. Troponin levels rise even in instances of minimal cellular injury, making it a very specific and sensitive marker in this context (15).

Myocardial siderosis is the leading cause of mortality in people with β-thalassemia major because it can lead to iron overload cardiomyopathy, which is caused by inadequate erythropoiesis, hypoxia, and prolonged anemia (16). These individuals are, therefore, more susceptible to ischemia. Troponin is released when cell injury and the loss of myocyte contraction force occur (14).

A positive correlation was observed between the levels of Klotho and Troponin-I in β-TM patients' group, suggesting a direct and strong relationship between these two markers.

the α-klotho protein plays a multifaceted role in protecting against acute cardiorenal injury, reducing myocardial stress, regulating oxidative stress, Suppression of tissue fibrosis following inflammatory conditions, The control of blood pressure reaction to sodium chloride intake, the ability to prevent cardiac hypertrophy, Prevention of coronary artery disease, Stroke prevention, Reduction in blood pressure, and Avoidance of arterial calcification. These actions of Klotho contribute to its overall cardioprotective effects (17).

In conclusion, the study reported an increase in Klotho protein and troponin levels among beta thalassemia major patients in Iraq, with a notable positive correlation between them. These findings suggest a potential relationship between Klotho, known for its cardioprotective properties, and cardiac injury markers like troponin in this patient population.

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Conflict of interest

There is no Conflict of interest.

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