



RESEARCH ARTICLE

The Level of Heart-Type Fatty Acid Binding Protein (H-FABP) as Risk Marker for Cardiac Dysfunction among Some Beta-Thalassemia Major Patients in Baghdad City-Iraq

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ARTICLE INFO	ABSTRACT
Received: May 22, 2024 Accepted: Jul 5, 2024	Beta thalassemia major (β -TM) is a prevalent genetic disorder due to a lack of globin chains. Heart-type fatty-acid binding protein (H-FABP) is a small protein in the cytoplasm released into the bloodstream when heart muscle cells are injured. H-FABP is regarded as a highly responsive indicator of continuous harm to the heart muscle and has the ability to predict cardiovascular problems in the overall population. This study aims to determine whether serum H-FABP levels in patients with thalassemia are significant and whether its concentration correlates with serum ferritin levels and cardiac enzymes. 60 subjects were included in this study to measure their serum H-FABP. 30 Beta-Thalassemia Major (β -TM) patients, none of them have symptoms of heart dysfunction, and 30 normal subjects were enrolled in this study. Serums H-FABP, Troponin-I, and BNP were determined using the ELISA technique in addition to serum Ferritin by a Clinical Automation system (Beckman Coulter). Serum H-FABP, Troponin-I, BNP, and Ferritin increased in β -TM patients compared to control healthy subjects ($P < 0.001$). A negative correlation was found between serum H-FABP and Troponin-I in the patients' group. Elevated level of H-FABP and a negative correlation was found between serum H-FABP and Troponin-I in the β -TM Patients.
Keywords Beta-Thalassemia Major H-FABP Troponin-I BNP Cardiac dysfunction.	
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INTRODUCTION

Thalassemia is a prevalent global condition characterized by the fast loss of red blood cells. To sustain red blood cell levels, patients must undergo regular blood transfusions. Regular blood transfusions can result in iron excess, which can subsequently lead to consequences such as heart disease, diabetes, osteoporosis, and renal issues(1).

β -thalassemia patients experience cardiac impairment mainly due to left ventricular (LV) dysfunction, which gradually results in heart failure and, ultimately, mortality(2).

Ferritin is a protein that stores iron in the body to protect cells from the harmful effects of excessive metal levels. Research has additionally demonstrated that ferritin is responsible for storing iron and converting excess iron into hydrous ferric oxide deposited within its cavity. Ferritin's capacity to

sequester iron enables it to play two distinct roles: detoxification and preserving the cellular iron reserve(3).

Troponin (Tn) is a crucial intracellular protein that regulates contraction in striated muscles. The discovery of this protein occurred in 1965 and was first referred to as "tropomyosin-like protein" until 1973. At that time, it was isolated and identified from the skeletal muscles of a rabbit as a distinct complex composed of three distinct protein subunits. Troponin (Tn) is a protein composed of three subunits (C, T, and I) with varying molecular weights and specific activities(4).

B-type natriuretic peptide (BNP) is a cardiac hormone secreted by the myocardium of the left ventricle in response to increased pressure or volume in the heart. BNP stimulates sodium excretion and causes the narrowing of blood vessels to control the amount of blood and the pressure within it. Elevated BNP levels are observed as the left ventricular function worsens(5).

Heart-type fatty acid-binding protein (H-FABP) is more highly expressed in the heart's ventricles and atria than in skeletal muscles or other organs. H-FABP is promptly released from cardiac muscle cells into the bloodstream following damage to the heart because of its compact size and unrestricted presence in the cell's cytoplasm. Furthermore, it is likely to have a crucial function in many auto- and paracrine pathways involved in the development of HF. H-FABP served as a reliable signal of cellular destruction and a marker of abnormal functioning and dysfunction of the heart muscle, leading to impaired myocardial function(6).

MATERIALS AND METHODS PATIENTS

Patients and control

The samples collected at the beginning of the study were 60 subjects selected with an age range (18-30 years) living in Baghdad; each patient completed a questionnaire sheet that included the following information: code number, name, age, gender, date, address, ethnicity, family history of thalassemia, weight, length, and medical history. This study was performed in the Ibn Albaladi Center of Blood Diseases (during the period from 1st of March 2023 to the end of August 2023). These subjects were divided into two groups: 30 Beta-Thalassemia Major patients, none of whom had heart dysfunction symptoms, and 30 normal subjects. Patients with cardiovascular disease were excluded in this study. Consent has been acquired from all patients and healthy volunteers, or their parents, for this study, and it was publically acknowledged.

Statistics

Continuous data were described as mean± SD (Standard Deviation). 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

Blood sampling

Blood samples were collected from subjects at 8:00 a.m.- 11:00 a.m. The specimen was taken from the vein and preserved using a disposable syringe with a capacity of 10 ml. The sample was kept in dispensable tubes containing a gel, facilitating serum separation processes.

The gel tubes containing blood were incubated at 37°C for roughly 10 -15 minutes until clotting occurred. Subsequently, the tubes were centrifugated at 2000 (Xg) for 10 to 15 minutes. Next, the serum was divided and preserved at a temperature of -20°C using sterilized Eppendorf tubes. 0.5ml of serum was used until analysis Serums H-FABP, Troponin-I, BNP and Serum Ferritin.

Determination of Serum H-FABP, Troponin-I, BNP by enzyme-linked immunosorbent assay (ELISA) kits which are sandwich enzyme immunoassay for in vitro quantitative measurement H-FABP-SEB243Hu Cloud-Clone Corp(USA)], [BNP- CEA541Hu Cloud-Clone Corp(USA)], [Troponin-I - SEA478Hu Cloud-Clone Corp(USA)], and serum Ferritin by Clinical Automation system (Beckman Coulter).

RESULTS

Demographic characteristics of the β -Thalassemia major 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.

In Table (1), the Mean \pm SD for age across the groups was statistically similar, as indicated by the p-value of 0.987. Figure 1

There is a significant difference in BMI across the groups with p-value <0.01 β -TM group had a Mean \pm SD (21.12 \pm 0.95 Kg/m²), and the Control group had a Mean \pm SD (23.72 \pm 1.41 Kg/m²). Figure 2

Table 1: Demographic and Laboratory data among β -Thalassemia Major (β -TM) and Controls groups

parameter	Controls (n = 30)	β -Thalassemia Major (β -TM) (n = 30)	P value
Male	17/30	17/30	1
Female	13/30	13/30	1
Age (Years)	22.83 \pm 2.03	22.67 \pm 4.78	0.987
BMI Kg/m ²	23.72 \pm 1.41	21.12 \pm 0.95	<0.001
Ferritin ng/mL	53.20 \pm 17.01	4703.17 \pm 3390.18	<0.001
BNP (pg/mL)	96.63 \pm 16.13	361.63 \pm 74.72	<0.001
Troponin (pg/mL)	436.67 \pm 163.48	1472.10 \pm 499.59	<0.001
H-FABP (ng/mL),	12.82 \pm 1.02	24.80 \pm 3.87	<0.001

Note: Each parameter's mean and standard deviation (Mean \pm SD) are provided, along with the p-value indicating the significance of the differences between the groups.

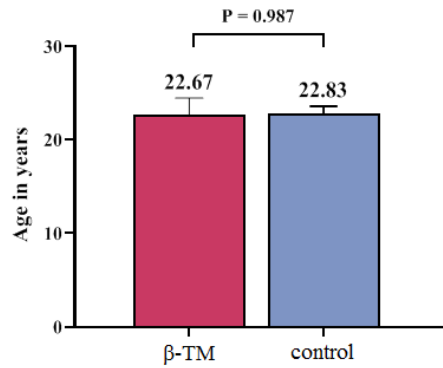


Figure 1: Means of age by Groups with 95.00% CI Error Bars

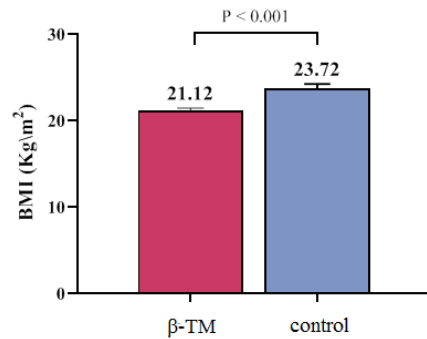


Figure 2: Means of BMI by Groups with 95.00% CI Error Bars

There was a significant increase in serum ferritin in the β -TM patients group (4276.73 ± 2401.39 (ng/mL)) against the control group (53.20 ± 17.01 (ng/mL).). Figure 3

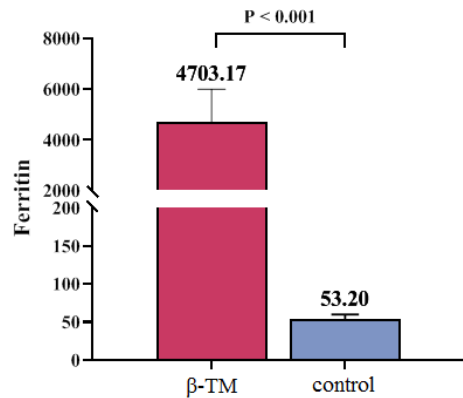


Figure 3: Means of Ferritin by Groups with 95.00% CI Error Bars

For Troponin, the Mean \pm SD values for the β -TM group and Control group are Mean \pm SD (1472.10 \pm 499.59 pg/mL) and (436.67 \pm 163.48pg/ML), respectively. Figure 4

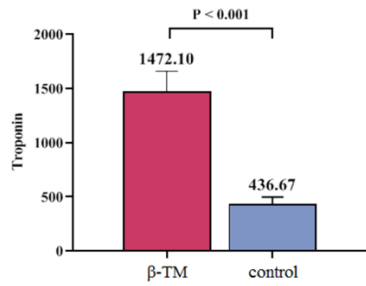


Figure 4: Means of Troponin by Groups with 95.00% CI Error Bars

In this study, it was shown that BNP levels were elevated in the β -TM group had Mean \pm SD (361.63 \pm 74.72 pg/mL) compared with the healthy control group had Mean \pm SD (96.63 \pm 16.13 pg/mL). Figure 5

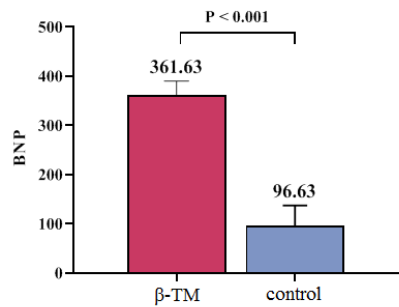


Figure 5: Means of BNP by Groups with 95.00% CI Error Bars

Lastly, a significant difference with p-value < 0.001 between β -Thalassemia Major (β -TM) and the control group for S.H-FABP, the Mean \pm SD values for (β -TM) group (24.80 \pm 3.87 ng/mL) and the control group had Mean \pm SD (12.82 \pm 1.02 ng/mL). Figure 6

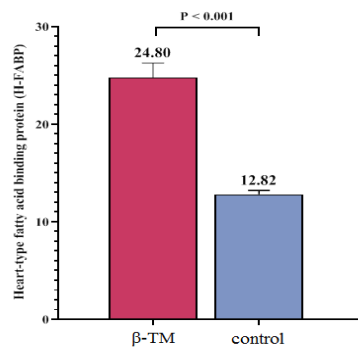


Figure 6: Means of H-FABP by Groups with 95.00% CI Error Bars

Negative correlation exists between H-FABP and Troponin-I (coefficient of -0.49), suggesting an inverse relationship between these two variables.

Table 2: Correlation matrix (Pearson) / β -Thalassemia Major (β -TM) group

Variables	Troponin	BNP	H-FABP	Ferritin
Troponin	1	-0.12	-0.72	0.24
BNP	-0.12	1	0.32	-0.05
H-FABP	-0.72	0.32	1	-0.11
Ferritin	0.24	-0.05	-0.11	1

DISCUSSION

There is a significant difference in BMI across the groups with p-value <0.01 which is approximately similar to the finding of Talib et al.,(7)who reported a BMI β -TM patients was lower than control.

The etiology of inadequate weight gain, low BMI, and disrupted body composition seems to be multifactorial and primarily stems from nutritional insufficiency (both macronutrients and micronutrients), persistent anemia and hypoxia, excessive iron accumulation in various organs, insufficient utilization of chelating agents, and endocrine disorders (such as hypogonadism, delayed puberty, hypothyroidism, and dysregulation of the GH-IGF-1 axis)(8).

There was a significant increase in serum ferritin in the β -TM patients group this agrees with several previous studies(7)(9)

This excessive accumulation of iron typically occurs due to blood transfusion and insufficient production of red blood cells. In individuals with thalassemia, mutations lead to the production of the GDF15 protein, which acts as an inhibitor of the peptide-hepcidin hormone. This protein sends a signal to the liver, causing a reduction in hepcidin levels. Ferroportin enhances iron absorption from the diet by reducing Hcpidin levels. Consequently, when there is a lack of red blood cell production in the spleen, they become trapped, causing the release of iron, ultimately leading to an elevation in ferritin levels(10).

There was a significant increase in serum Troponin-I in the β -TM patients group than the control group. these results are consistent with Saeed, (11). 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, prolonged anemia, and hypoxia. These individuals are, therefore, more susceptible to ischemia. Troponin is released when cell injury and the loss of myocyte contraction force(11).

The leading causes of cardiomyopathy in patients with thalassemia are increasing intestinal absorption of iron, hemolysis, and lifelong blood transfusions. When intracellular iron increases, it is metabolized, releasing reactive oxidative species, which damage the cell membrane and interfere with the respiratory chain in the mitochondria, resulting in cardiotoxicity(12).

In this study, it was shown that BNP levels were elevated in the β -TM group compared with the healthy control group. These results are similar to those reported by (Mohammed et al.) and exhibit significant differences with p -value < 0.001 (2).

The most important complications in patients with beta-thalassemia major are cardiomyopathy and various types of arrhythmias. In patients with beta-thalassemia, major anemia leads to increased cardiac output that results in left ventricular hypertrophy and ends in heart failure. Iron overload will result in peroxidation and cellular injuries due to iron overload that causes left ventricular cardiomyopathy. Also, cardiac arrhythmia like atrial fibrillation, ventricular tachycardia, and supraventricular tachycardia are increased according to increased cardiac siderosis(13).

The present study shows that serum H-FABP of patients with the beta-thalassemia major group was significantly higher than that of the control group. These results were in agreement with Elmalah, (14) who found in his study that patients with thalassemia major who did not previously experience any manifestation of cardiac dysfunction have higher serum H-FABP than healthy children.

Research has shown that several factors may contribute to increased H-FABP levels. Firstly, Beta thalassemia results in abnormal or decreased production of hemoglobin and iron overload, leading to hemolysis and chronic anemia(15). This leads to increased cardiac output, impaired blood flow, and oxygen delivery to the heart, potentially resulting in cardiac injury and elevated H-FABP levels(16).

secondly, Thalassemia patients often require frequent blood transfusions, which can lead to iron overload in the body, including the heart. Cardiac iron overload can cause oxidative stress, inflammation, and damage to heart cells(17), releasing H-FABP into the bloodstream.

Negative correlation exists between H-FABP and Troponin-I (coefficient of -0.49), suggesting an inverse relationship between these two variables.

H-FABP exists as a soluble protein within the cytoplasm. Consequently, the entry of the substance into the bloodstream can be identified more quickly, even following slight damage to the heart muscle. The study conducted by Liebetrau et al (18) found a notable rise in blood levels of H-FABP within fifteen minutes following an iatrogenic myocardial infarction(6) whereas troponin release occurs later. Therefore, in the early stages of myocardial injury, when H-FABP levels are elevated, troponin levels may still be within the normal range. As the injury progresses and more cardiac muscle damage occurs, troponin levels increase while H-FABP levels may decrease or plateau.

CONCLUSION

A higher concentration of Heart Fatty Acid Binding Protein (H-FABP) in thalassemia patients compared to a healthy control group may suggest the presence of heart complications in thalassemia. These findings indicate that individuals with thalassemia may have a higher susceptibility to cardiovascular problems than the overall population. Measuring H-FABP concentration with other cardiac enzymes and following up on its results may show the development of heart problems in thalassemia patients in their early stages before reaching higher stages.

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