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Original Research Article

Biomarkers of Myocardial Dysfunction in Children with B-Thalassemia Major: Controlled Study

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Abstract

Background: Children with ß-thalassemia major are at higher risk for myocardial dysfunction that adversely affects their quality of life. Cardiac biomarkers are valuable tool for assessment of such children. Our aim was to assess heart fatty acid-binding protein (H-FABP) versus B-type natriuretic peptide (BNP) in thalassemia patients as biomarkers of myocardial dysfunction. ***Methods:*** This controlled study involved 35 children with ß-thalassemia major and 35 healthy children. Clinical assessment, echocardiography, serum ferritin, BNP and H-FABP were done. ***Results:*** H-FABP and BNP were higher in thalassemia than healthy children. Left ventricular dimension and pulmonary pressure were significantly higher and ejection fraction was significantly lower in thalassemia patients. H-FABP has positive correlation with BNP, left ventricular dimension, pulmonary pressure, serum ferritin and the duration of blood transfusion. ***Conclusion:*** Elevated H-FABP is associated with myocardial dysfunction in otherwise asymptomatic thalassemia patients.

Keywords: H-FABP, Thalassemia**,** Echocardiography.

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INTRODUCTION

Children with ß-thalassemia major are exposed to several risk factors of myocardial dysfunction including chronic anemia and regular blood transfusions causing iron overload cardiomyopathy. Cardiac complications are a leading cause of morbidity and mortality among those children that adversely affect their quality of life [1].

Cardiac impairment in ß-thalassemia patients characterized mainly by left ventricular (LV) dysfunction which leads progressively to heart failure and finally death [2]**.** Echocardiography is the gold standard for assessment of cardiac function however; advanced modalities including 4D and speckle trickle techniques are expensive, need experts and not widely available. Using cardiac biomarkers are extensively studied and evidences revealed valuable diagnostic and prognostic role of several markers when combined with conventional echocardiography [3].

B-type natriuretic peptide (BNP) is a cardiac hormone that released from the left ventricle myocardium in response to either pressure or/ and volume overload. BNP acts by induce natriuresis and peripheral vasodilatation to regulate blood volume and blood pressure. BNP release is elevated as left ventricular function deteriorates [4].

Fatty acid-binding proteins are tissue specific intracellular molecules of about 15 kD. They are cytoplasmic proteins that bind long chain fatty acid and play an important role in the intracellular utilization of fatty acids. H-FABP and other types of FABP are different in morphology and immunology, cross-reaction does not occur, so H-FABP is highly specific for the diagnosis of myocardial injury [5].

H-FABP released rapidly into the circulation after myocardial injury. Serum H-FABP levels increase in children with chronic heart disease and are closely related to the progression of the underlying pathological condition. Serum H-FABP can be used as biomarker for the diagnosis myocardial dysfunction and evaluation of its severity [6]. However, its role for assessment of cardiac affection in children with thalassemia is not fully explored yet. This study aimed to assess serum H-FABP in children with ß-thalassemia major and their relation with serum ferritin and echocardioghraphic changes.

PATIENTS AND METHODS

Study Design and Participants

This study is an observational controlled study included 70 children their age ranged from 10-15 years. They were categorized into 2 groups; 35 child with ß-thalassemia major as patient group and 35 ages and gender matched healthy child as control group. Children were considered illegible if they have confirmed diagnosis of ß-thalassemia major on regular blood transfusion who were referred for echocardiography at Al-Zahraa University hospital, Cairo, Egypt during the period from May 2018 to September 2018. An informed consent was received from caregivers of all included children and the study was approved by the local ethics committee of Al-Azhar University.

Exclusion criteria included children with congenital, rheumatic heart disease, heart failure or any significant cardiac disease, children with chronic kidney disease hematologic disorder other than thalassemia, children with acute infection.

Medical History and Examination

All included children subjected to full history taking according to pre designed questionnaire with stress on age of onset of anemia, onset and frequency of blood transfusion, type of chelation therapy, family history of similar condition, splenectomy and cardiac symptoms. Complete general and systematic examination was done with stress on vital signs (blood pressure, heart rate and respiratory rate), anthropometric measures (weight, height and BMI), **c**ardiac and abdominal examination.

Laboratory Investigations

5ml of venous blood was withdrawn and divided into 2specimens. 3 ml blood was added to plan tube and left to clot in water bath (37C°) for 30 min, then centrifuged at 2000 rpm for 15 minutes. Then the serum collected and divided into 2 aliquots. One was used for measuring ferritin by Enzyme Immunoassay and C-reactive protein by latex serology test. The second aliquot was stored at –20C° for subsequent measuring of H-FABP and BNP using enzyme-linked immunosorbent assay (ELISA) according to the manufacture provided by Elabscience Biotechnology Co., Ltd. 2ml of blood was added to EDTA tube for measuring complete blood picture using (Coulter Counter, Beckman Inc, Florida, USA) and erythrocyte sedimentation rate using Westergren method

Measurement of serum H-FABP

The H-FABP quantitative Test is based on a solid phase. The assay system utilizes an affinity purified goat anti-H-FABP antibody for solid phase (microtiter wells) immobilization and the same goat anti-H-FABP antibody in the antibody-enzyme (horseradish peroxidase conjugate solution. Reading was done by using ELISA Tecan sunrise reader adjusted at 540 nm and 570 nm for background .

Measurement of serum BNP

The BNP quantitative Test is based on the principle of competitive enzyme immunoassay using precoated microplate with anti-rabbit secondary antibody. The plate was incubated with anti-BNP antibody where biotinylated BNP peptide and standard peptide react competitively with the BNP antibody then the bounded biotinylated BNP peptide interacted with Streptavidin-horseradish peroxidase producing a color reaction That inversely proportionate to the amount of BNP.

Transthoracic echocardiographic examination (TTE)

Transthoracic two dimensional (2D) guided (M Mode) and color Doppler echocardiographic examination was performed for all children and control subjects in both supine and left lateral position using a GE system Vivid-7, with matrix probe M3S multi frequency 2.5 MHZ. Patient's recordings are taken while patients are in supine position without breath holding. M-mode, 2D and Doppler echocardiographic parameters are averaged over 3 cardiac cycles.

Two dimensional echo images were obtained from the parasternal (long and short axis), subcostal and apical views (apical four and five chambers) to assess the anatomy and integrity of valves**.**

The LV dimensions were obtained from 2D echo guided M-Mode from parasternal long axis view and short axis view at the level of the papillary muscle to assess: left ventricular end diastolic dimension (LVEDd), left ventricular end systolic dimension (LVESd), left ventricular ejection fraction (EF%) according to this formula**:** LVED - LVES volume/ LVED volume. LV systolic dysfunction in children was defined if ejection fraction (EF) <55% [7].

Right ventricular dimentions were taken from two dimentions M-Mode at para sternal long axis views at the level of left ventricular papillary muscle.

Pulmonary artery flow was obtained by posterior wall Doppler examination guided by Doppler color flow with the sample volume placed just distal to the pulmonary valve in the short axis view at the level of great vessels with measurement of pulmonary flow acceleration time.

Pulmonary artery systolic pressure (PASP) calculation in the presence of tricuspid regurgitation, using color flow guided CW Doppler to get the maximum systolic pressure gradient (PGs) throughout the valve and adding 10 mmHg [8].

Statistical Analyses

Data were analyzed using Statistical Package for Social Science (SPSS) version 22. Demographic & clinical data were presented as mean values ± standard deviations or as percentages. Weight, height, right and

left ventricular dimensions were expressed in terms of standard deviation score (Z-score). Difference between two groups was compared by independent t-test and chi-square test. Pearson's correlation coefficient was used to determine correlations between two sets of data of different variables. Receiver operating characteristic curves (ROC) were used to identify sensitivity, specificity and determine optimal cut-off points of H-FABP and BNP to detect left ventricular dysfunction (EF%<55). Significance level was taken at p value ≤ 0.05.

RESULTS

Among the 35 children with thalassemia major who participated in this study 21 were males (60%) and 14 were females (40 %). Control group comprised 35 children, 22 males (62.8 %) and 13 females (37.2 %).

The age of clinical presentation in children with thalassemia major ranged from 6-24 months old and their duration of illness ranged from 1-14.5 years. The duration of blood transfusion ranged from 1-14.5 years and the frequency of transfusion ranged from 1-4 times/ month. At the time of enrollment in the study, splenectomy was done for 45% of children with thalassemia. Positive family history of thalassemia was found in 60% of children with thalassemia major. Among the 35 thalassemic patients 51.4% of them receive deferasirox as iron chelation therapy and 20% receive deferiprone. Adjuvant parenteral iron chelator deferoxamine was used in 20% of the studied children. While, 20% of them did not receive any iron chelation therapy**.** None of the included children have symptoms suggestive of heart failure.

In comparison to healthy controls, children with thalassemia major have significant lower weigh, height, BMI and EF% and significant higher right and left ventricular dimension, EPASP, serum ferritin, BNP and H-FABP level as demonstrated in Table-1.

Table-2 showed the correlation between clinical and echocardiographic data of children with thalassemia major and serum level of H-FABP.

Receiver operating characteristic curves (ROC) demonstrated that At cutoff point >21.5 ng/ml H-FABP has sensitivity of 93.3% and specificity of 99.5% for prediction of left ventricular dysfunction while BNP has sensitivity of 93 % and specificity of 100% at cutoff point >31ng/ml as shown in Table-3, Figure-1.

Table-1: Comparison of children with thalassemia and healthy controls regarding clinical, biochemical and echocardiographic findings

	Thalassemia group (n = 35)	Control group (n = 35)	Independent T test/ Chi-square test	
	Mean ± SD	Mean ± SD	x^2/t	P-value
Age (years)	12.152 ± 2.283	12.392 ± 2.455	0.153	0.847
Sex (Number, %) Male Female	21 (60%) 14 (40%)	22 (62.8%) 13 (37.2%)	0.047	0.829
Weight (Kg) Z-score	-0.280 ± 0.651	0.282 ± 1.291	-2.640	0.014*
Height (cm) Z-score	-0.240 ± 0.942	0.283 ± 1.02	-2.364	0.033*
BMI Z-score	-0.240 ± 1.081	0.272 ± 1.651	-2.219	0.042*
Serum ferritin (ng/dl)	234.821 ± 1071.523	68.172 ± 28.991	11.786	<0.0001*
Serum BNP (ng/ml)	33.725 ± 12.985	9.281 ± 3.726	12.986	<0.0001*
H-FABP (ng/ml)	22.632 ± 12.086	6.343 ± 3.182	9.625	<0.0001*
LVEDD (mm) Z-score	0.408 ± 1.077	-0.408 ± 0.722	4.227	<0.0001*
LVESD (mm) Z-score	0.368 ± 0.985	-0.368 ± 0.880	3.742	<0.0001*
EF%	65.821 ± 8.365	77.932 ± 9.844	-4.172	<0.0001*
RVD (mm) Z-score	0.003 ± 1.159	-0.003 ± 0.823	0.033	0.974
PASP (mmHg)	37.375 ± 5.862	21.027 ± 3.373	10.203	<0.0001*

*significant; PASP: Pulmonary artery systolic pressure; RVD: right ventricular dimension; LVEDD: left ventricular end diastolic dimension; LVESD: left ventricular end systolic dimension; EF: ejection fraction; H-FABP: heart type fatty acid binding protein; BNP: B-type natriuretic peptide

Table-2: Comparison of cardiac biomarkers between thalassemia children with preserved and impaired ejection fraction with controls

	Thalassemia with preserved EF% N=20	Thalassemia with impaired EF% N=15	Healthy controls N=35	f	One-way ANOVA
Serum BNP (ng/ml)	24.947 ± 3.605	35.4667 ± 4.661	9.281 ± 3.726	22.879	<0.0001*
H-FABP (ng/ml)	17.750 ± 1.551	24. 200 ± 3.605	6.34 ± 3.18	25.982	<0.0001*
Post hoc analysis					
	Thalassemia with preserved EF% vs control	Thalassemia with impaired EF% vs with control	Thalassemia with preserved EF% vs with impaired EF%		
Serum BNP (ng/ml)	<0.0001*	<0.0001*	<0.0001*		
H-FABP (ng/ml)	<0.0001*	<0.0001*	<0.0001*		

*significant; EF: ejection fraction; H-FABP: heart type fatty acid binding protein; BNP: B-type natriuretic peptide

Table-3: Correlation between serum H-FABP and echocardiographic finding in children with thalassemia

	H-FABP	
	r	p-value
Age (years)	0.437	0.001*
Age of first transfusion (months)	-0.108	0.698
Duration of blood transfusion (year)	0.502	<0.0001*
Serum ferritin (ng/dl)	0.639	<0.0001*
Serum BNP (ng/ml)	0.527	<0.0001*
LVEDD (mm) z-score	0.477	<0.0001*
LVESD (mm) z-score	0.425	<0.0001*
EF%	-0.331	0.001*
RVD (mm) z-score	0.311	0.003*
EPASP (mmHg)	0.421	<0.0001*

*significant; EPASP: Estimated Pulmonary artery systolic pressure; RVD: right ventricular dimension; LVEDD: left ventricular end diastolic dimension; LVESD: left ventricular end systolic dimension; EF: ejection fraction; H-FABP: heart type fatty acid binding protein; BNP: B-type natriuretic peptide

Table-4: senstivity and specificity of H-FABP and BNP for prediction of myocardial dysfunction in thalassemia children

variables	Cutoff	AUC	Senstivity%	Specificity%	95% Confidence Limits
BNP (ng/ml)	>31	0.992	93%	100%	0.912 – 1.000
H-FABP (ng/ml)	>21.50	0.997	93.33%	99.5%	0.972 – 1.000

AUC: area under curve; H-FABP: heart type fatty acid binding protein; PAPP-A: pregnancy associated plasma protein-A

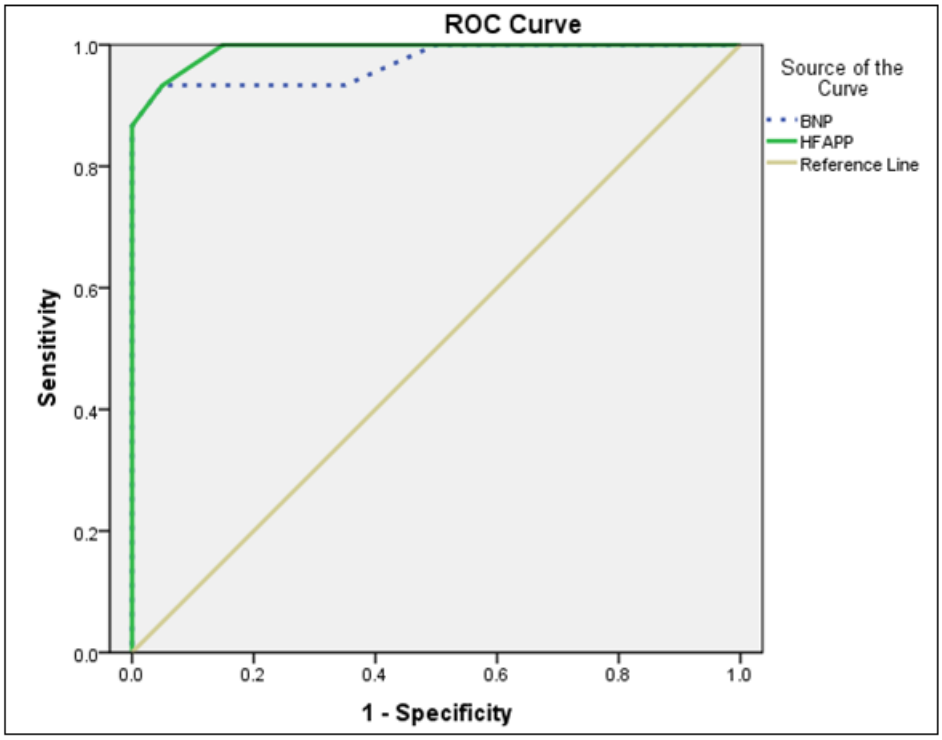

Fig-1: Receiver operating characteristic curves for prediction of myocardial dysfunction in thalassemia children

DISCUSSION

Echocardiographic examination revealed that left ventricle was significantly impaired in asymptomatic children with thalassemia. Our findings agree with previous reports that transfusion-related myocardial dysfunction including left ventricular systolic and diastolic dysfunction, pulmonary hypertension, arrhythmias and pericarditis are The most common cardiac abnormalities in patients with thalassemia major [9]. Ibrahim *et al*., [10] concluded that the earliest signs of dilated cardiomyophathy in echocardiography are increased LV dimensions. Cardiac dysfunctions in thalassemia major have attributed to chronic anemia, infrequent transfusions, iron-overload and inadequate chelation therapy [11]. In addition to pulmonary vascular load caused by diastolic dysfunction, chronic hypoxia, free iron, phosphatidylserine-expressing hematologic debris are a powerful stimulus for vasoconstriction [12]. This data could explain significant higher pulmonary artery systolic pressure in our studied thalassemia patients.

Several cardiac biomarkers are used for early detection of myocardial dysfunction in children with thalassemia. However no strong evidences suggest any marker to be better than others. In agreement with our findings, Kremastinos *et al*., [13] demonstrated that BNP is significantly higher in children with thalassemia especially those with myocardial dysfunction.

H-FABP is a sensitive and specific biomarker of myocardial injury that rapidly released from cardiomyocytes into the circulation after the onset of cell damage [14]. H-FABP is superior to traditional biomarkers for the assessment of recurrent or persistent myocardial damage impairment in several categories of patients with heart diseases; however little information are available regarding the clinical usefulness of this marker in thalassemia patients. An elevated H-FABP identified patients at risk for death and major cardiac events even when troponins and CK-MB are not elevated [15]**.** Sun *et al*., [16] reported that H-FABP is sensitive to ongoing myocardial damage and can identify patients who are at high risk for heart failure. Ozdemir *et al*., [17] demonstrated that H-FABP is an earlier marker for myocardial injury than serum creatine kinase iso enzyme MB or cardiac troponin T.

In our study, children with thalassemia major who did not previously experienced any manifestation of cardiac dysfunction have higher serum H-FABP than healthy children. Additionally, H-FABP has significant correlation with left ventricular dimension and its elevation is strongly correlated to decreased EF%.

In the current study, growth parameters for weight and height were significantly lower for thalassemia children in comparison to healthy control group which agree with previous reports [18, 19]. Children with thalassemia are at high risk growth impairment due to the effect of chronic anemia, transfusion iron overload, endocrinopathy, and chelation toxicity [20]. To avoid the variation in cardiac champers dimensions related to differences in body surface area between children with thalassemia and healthy controls so right and left ventricular dimension were expressed as Z-scores.

Among the studied children with thalassemia, 20% did not receive any iron chelation therapy. This reflects poor compliance and unsatisfactory chelation therapy among our studied patients that make them at high risk for iron over load complication.

As a sequence of hemolysis, ineffective erythropoeisis, increased iron absorption and transfusion therapy; our studied thalassemia children have statistically higher serum ferritin level than healthy children. Serum ferritin is used as indirect measure for iron over load among these children. However serum ferritin level did not reflect cardiac iron deposition [21]. Cardiac magnetic resonance is the preferred technique for quantifying cardiac iron deposition. However, it is higher cost and not feasible or available for routine clinical practice. Conventional two-dimensional echocardiography remains a clinically useful method for evaluation and follows up of thalassemia patients [22].

Iron overload cardiomyopathy represents an ongoing, chronic, and progressive process with aging that can occur in subjects receiving long term blood transfusion. These could explain the significant positive correlation between patient age, duration of blood transfusion and serum level of H-FABP in our patients in addition to the significant positive correlation between H-FABP and serum ferritin.

Myocardial damage plays a key role in the progression of left ventricular remodeling in heart failure. H-FABP is a sensitive marker for detecting latent myocardial damage and is a useful marker for detecting subjects at high risk for developing heart disease. H-FABP level can reflect the progression of cardiac remodeling and cardiomyocytes metabolic activity as well as on the pathogenesis of cardiomyopathy [23]. This is in accordance with our finding of statistically significant positive correlation between serum H-FABP level and echocardiographic parameters of left and right ventricular dimensions.

Our study demonstrated that H-FABP has higher sensitivity than BNP to predict impaired left ventricular dysfunction. This agrees with previous report by Niizeki *et al*., [24] who found that FABP is superior to BNP for predicting cardiac events in patients with chronic heart failure.

CONCLUSION

Elevated H-FABP is associated with myocardial dysfunction in otherwise asymptomatic thalassemia patients. Longitudinal studies are required to evaluate its prognostic role in children with thalassemia.

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