Serum ferritin as a diagnostic marker for cardiac iron overload among beta-thalassemia major children

Subash Chandra Majhi[1], Nihar Ranjan Mishra[2], Prakash Chandra Panda[1], Sumeet Soumyaranjan Biswal[3]

From[1] Associate Professor, [2]Assistant Professor, [3]Postgraduate Student, Department of Pediatrics, Veer Surendra Sai Institute of Medical Science and Research, Burla, Sambalpur, Odisha, India

Correspondence to: Dr. Nihar Ranjan Mishra, Department of Pediatrics, Veer Surendra Sai Institute of Medical Science and Research, Burla, Sambalpur, Odisha, India. E-mail: drnihar.mishra@gmail.com

Received - 19 March 2019 Initial Review - 08 April 2019 Accepted - 18 April 2019

ABSTRACT

Introduction: Beta-thalassemia major is the most common chronic hemolytic anemia. It is a well-comprehended fact that the toxic effects of iron overload particularly the cardiomyopathy are the major complication that roots from beta-thalassemia major children. Therefore, timely diagnosis is crucial to optimize the long-term gain. **Objective:** The objective of the study is to find the cutoff level of serum ferritin for early diagnosis of cardiac iron overload. **Materials and Methods:** This study was an observational analytical cross-sectional diagnostic study which was conducted from November 2016 to October 2018. With due approval of Institutional Ethics Committee and after taking proper informed consent from the parents and/or legal heir, 105 thalassemic children were enrolled in the study by simple consecutive sampling after satisfying the pre-defined inclusion and exclusion criteria. In this study, two-dimensional Doppler echocardiography was used to detect cardiac iron overload. Serum ferritin levels were estimated, and cutoff values were calculated for each of the echocardiographic parameters of cardiac iron overload, i.e. ejection fraction (EF), left ventricular end-diastolic diameter (LVEDD), and left ventricular end-systolic diameter (LVESD) by receiver operating characteristic curve analysis. Sensitivity (Sn), specificity (Sp), positive predictive value, and negative predictive value were calculated with considering $p<0.05$ as statistically significant. **Results:** The mean age of the study participants was 9±3 years. Cutoff value of serum ferritin for detecting abnormality in EF was 3286 ng/ml with Sn of 76.1% and Sp of 88.1%. Similarly, for detecting abnormal LVEDD, cutoff value of serum ferritin was 4640 ng/ml with Sn of 70.1% and Sp of 98.6%, and for LVESD, it was 3286 ng/ml with Sn of 90% and Sp of 70.5%. **Conclusion:** The serum ferritin level can be used as a reliable marker of myocardial iron overload among childhood beta-thalassemia and hence can be used as an important screening tool.

Key words: *Beta-thalassemia major, Ejection fraction, Iron overload, Left ventricular end-diastolic diameter, Left ventricular end-systolic diameter, Serum ferritin*

Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. Poorly treated patients are presented with growth retardation, anemia, icterus, poor weight gain, hepatosplenomegaly, ulcers, extramedullary hematopoiesis, and bony changes with bone marrow expansion. Repeated transfusion therapy leads to iron overload toxicity and systemic complications such as endocrine complications (stunting, sexual immaturity, diabetes mellitus, and pathology of the parathyroid, thyroid, adrenals, and pituitary), cardiomyopathy, and hepatic cirrhosis.

Heart failure secondary to iron overload is the main cause of death in patients with thalassemia major. Most of the patients suffer from myocardial fibrosis and cardiac dysfunction due to an overload of iron in cardiac tissues [1,2]. Iron chelation may reduce cardiac iron overload and reduce the rate of mortality in such patients. Heart injuries in iron overload cases include dilatation of the atria and ventricles, arrhythmia, valvular dysfunction, pericarditis, thickening of the muscles, and finally, heart failure [2,3]. Although frequent blood transfusion is routinely done, patients still suffer from chest pain, tachycardia, exhausting, and in some cases, sudden death. To have accurate iron chelation, it is necessary to measure the amount of a patient's iron. The most accurate method to measure iron level is liver biopsy; however, it is invasive and unable to provide an accurate measurement of heart iron level [4].

A great deal of interest has centered on the cardiac function that can be evaluated by echocardiography. Some studies have proved that serum ferritin cannot show total iron level, especially in the heart [5]. On the other hand, some researchers have agreed that serum ferritin is associated with the cardiac iron level [6]. In addition, they suggested that measurement of electrocardiographic parameters is useful methods for better evaluation of heart function. Other investigators showed that echocardiographic parameters such as ejection fraction (EF), left

ventricular end-diastolic diameters (LVEDD), and left ventricular end-systolic diameter (LVESD) fractional shortening were significantly associated with elevated serum ferritin level. We have planned the present study because all the previous studies have not derived any cutoff value of serum ferritin for early detection of cardiac manifestation.

METHODS

The present observational analytical cross-sectional diagnostic study (Phase II) was conducted from November 2016 to October 2018 in the Postgraduate Department of Paediatrics, of a tertiary care hospital in Burla, after taking approval from the institutional ethics committee. The sample size was based on correlation coefficient of EF with serum ferritin, i.e. r=0.3 as per previous study [6]. Sample size was calculated by regression correlation coefficient (testing for r=0) method with taking the r=0.3, and power of the study to be 90% and α=5%, and taking two-sided significance level, minimum sample size was calculated to be 104 using nMaster v2 (BRTC, Bagayam, Vellore).

Total 123 diagnosed (high-performance liquid chromatography) cases of multitransfused (received transfusion for >1 year and serum ferritin levels >/=1000 ng/ml) [7] beta-thalassemia major children between 6 and 14 years of age were screened for the inclusion in the study. Out of which, parents of 10 patients did not give their consent and 8 were excluded as per the pre-defined exclusion criteria. The exclusion criteria were patients with any pre-existing congenital heart diseases, rheumatic heart disease or any other structural heart defects, and the associated comorbidities such as hypertension, dyselectrolytemia, hyperthyroidism, and type 1 diabetes mellitus. Finally, 105 beta-thalassemic major children were enrolled for the study after taking written informed consent from their parents or legal heir by simple consecutive sampling technique.

Serum ferritin levels were measured using enzyme-linked fluorescent assay by Biomerieux, India. All the patients were investigated with two-dimensional echocardiography (as the gold standard) in the department of cardiology using the Philips HD11 model, and the 3 different parameters, namely EF, LVEDD, and LVESD, were noted for each of the patients. Normal mean EF is 66% (range: 56%–78%) [8]. Body surface area was calculated using nomogram [9], andbased on this, LVEDD and LVESD in mm were recorded.

All the data were collected, validated, and relevant statistical analyses such as estimation of sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), diagnostic odds ratio (DOR), and likelihood ratios were calculated with help of Dxt v1.0 software (BRTC, Bagayam, Vellore). Cutoff value of serum ferritin for each of the above three echo parameters was calculated by receiver operating characteristic curve analysis with SPSS v 24 (IBM, New York) and Dxt v1 software (BRTC, Bagayam, Vellore). For all statistical, $p<0.05$ was considered to be statistically significant.

RESULTS

The mean age of the study population was 9.5±3.2 years with the mean serum ferritin levels of 3245±1264 ng/ml and mean hemoglobin levels of 6.2±1.1 g/dl. In this study, out of 105 enrolled participants, 9 children were already under chelation therapy at the start of the study. The cutoff value of serum ferritin was 3261 ng/ml with optimum Sn of 76.1% and Sp of 93.2% (Fig. 1) for detecting abnormalities in EF (%) as evident by the area under the curve (AUC) of 84.5% (95% confidence interval (CI): 75.8–90.9%), p=0.001 and Youden index of 0.693. The cutoff value of serum ferritin was 4640 ng/ml with Sn of 68.75% and Sp of 100% (Fig. 2) for detecting abnormalities in LVEDD (mm) as evidenced by AUC of 79.7% (95% CI: 70.5–87.1%), p=0.001 and Youden index of 0.688. The cutoff value of serum ferritin was 3261 ng/ml with Sn of 90.0% and Sp of 68.42% (Fig. 3) for detecting abnormalities in LVESD (mm) as evidenced by AUC of 64.7% (95% CI: 54.1–74.0%), p=0.008 and Youden index of 0.584.

Based on these observations, we got the Sn of 76.1% (61.2–87.4%), Sp of 88.1% (77.1–95.1%), PPV of 83.3% (68.6–93.0%), NPV of 82.5% (70.9–90.9%), and DOR of 23.6 (8.35–66.87) to use serum ferritin as an early screening tool for the detection of EF abnormalities (Table 1). Similarly, we got the Sn of 68.8% (50.0–83.9%), Sp of 98.6% (92.6%–100%), PPV of 95.7% (78.1–99.9%), NPV of 87.8% (78.7–94.0%), and DOR of 158.4 (19.197–1307.0) to use serum ferritin as an early screening tool for detection of LVEDD abnormalities (Table 1). For detecting LVESD abnormalities, we got the Sn of 90.0% (55.5– 99.7%), Sp of 70.5% (60.3%–79.4%), PPV of 24.3% (11.8– 41.2%), NPV of 98.5% (92.1–100%), and DOR of 21.53 (2.6–178.1) to use serum ferritin as an early screening tool (Table 1).

From the above analysis, we have got the highest DOR of serum ferritin for detecting LVEDD abnormalities, which mean that the efficacy of serum ferritin for early detection of cardiac iron overload in terms of LVEDD was the highest, followed by

Table 1: Different diagnostic accuracy parameters of serum ferritin

Parameters	Sn (%)	Sp (%)	PPV (%)	NPV (%)	LR+[#]	LR-[##]	(DOR)[$]
Ser. fer_{LVEDD}*	70.1	98.6	95	86	50.18	0.31	158.4
Ser. fer_{EF}**	76.1	88.1	83	82	6.41	0.27	23.63
Ser. fer_{LVESD}***	90	70.5	24	98	3.05	0.15	21.5

***Different diagnostic accuracy parameters for serum ferritin levels in detecting cardiac dysfunction when LVEDD is the gold standard. **Different statistical parameters for serum ferritin levels in detecting cardiac dysfunction when EF is the gold standard. ***Different statistical parameters for serum ferritin levels in detecting cardiac dysfunction when LVESD is the gold standard [#]Positive Likelihood Ratio, [##]Negative Likelihood Rati, $Diagnostic Odd's ratio. EF: Ejection fraction, LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, PPV: Positive predictive value, NPV: Negative predictive value, DOR: Diagnostic odds ratio**

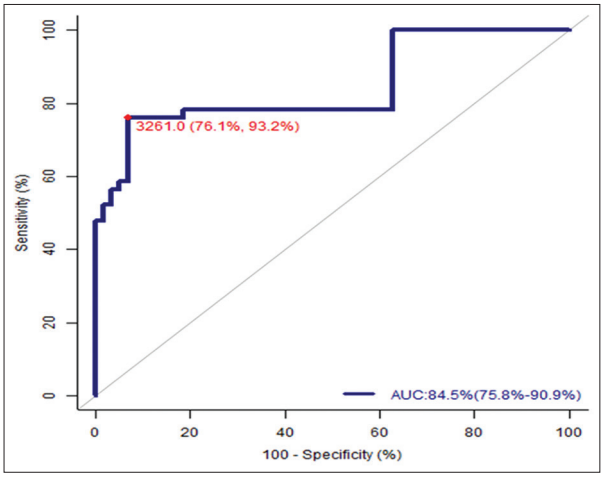

Figure 1: Receiver operating characteristic curve of serum ferritin for detecting abnormality in ejection fraction

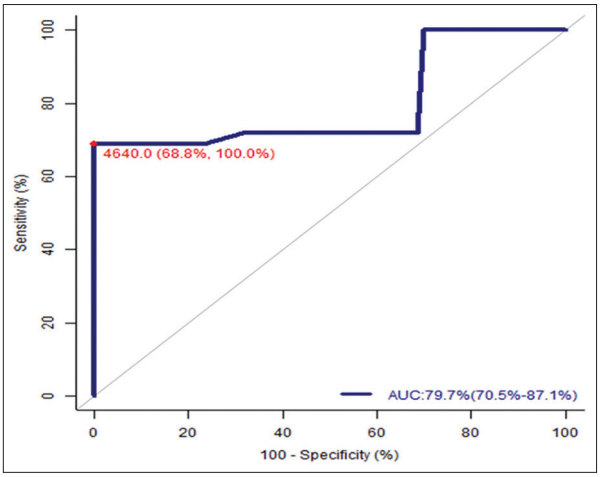

Figure 2: Receiver operating characteristic curve of serum ferritin for detecting abnormality in left ventricular end-diastolic diameter

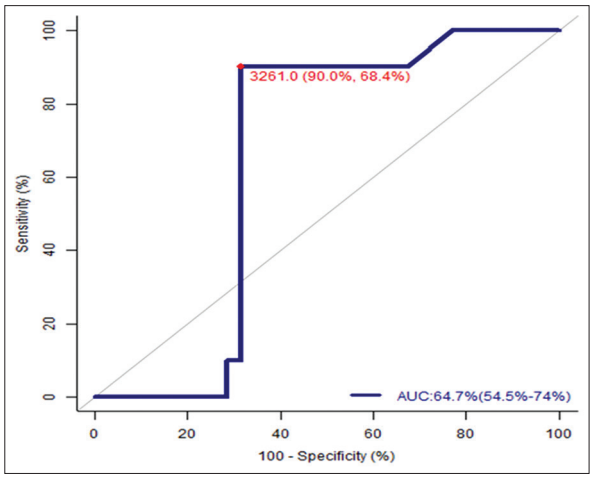

Figure 3: Receiver operating characteristic curve of serum ferritin for detecting abnormality in left ventricular end-systolic diameter

approximately same for EF and LVESD. The diagnostic accuracy was increased by 50 times if serum ferritin was used for detecting LVEDD abnormalities, but the accuracy decreases significantly if it was done for assessing EF and LVESD abnormalities.

DISCUSSION

The serum ferritin level is not specific for iron overload and may increase in many clinical conditions, including inflammation, liver disease, collagen tissue disease, and malignancy [11]. Moreover, a low serum ferritin level is not associated with a reduction in the risk of iron-induced cardiomyopathy [10]. Quick and easy detection of myocardial iron overload is crucial for detection of cardiac failure and its early management [12]. Proper treatment and early chelation therapy will lead to the early remediation [12,13]. In non-chelated patients receiving regular transfusions, cardiomegaly develops by the age of 10 years and heart failure by the age of 16 years [14]. Iron overload in thalassemia major (TM) occurs due to a combination of frequent blood transfusions and excessive gastrointestinal absorption [15]. Little is known about the natural history of iron deposition in the heart. Previous studies acknowledged that serum ferritin levels were higher in beta-thalassemia patients who had received more than 50 transfusions [16]. There are studies suggesting that myocardial deposition takes place after a minimum of 75 blood transfusions [15,16]. In one study of 110 patients echocardiographic abnormalities were found in patients with less than 50 transfusions [17]. Assessment of myocardial iron is essential clinically.

In another earlier reports, it was suggested that cardiac iron overload occurred only after at least 13 years of chronic transfusion therapy [18]. Serum ferritin level has been used as the most common predictor of iron overload in TM [19]. Echocardiography is useful to study anatomical changes of the heart; however, functional changes are usually observed in advanced stages of cardiomyopathy [20]. In one study, echocardiographic parameters such as the difference of pulmonary vein-atrial reversal flow and mitral valve A (PVAR-MVA) wave duration [21] and early ventricular filling velocity to early diastolic myocardial velocity ratio (E/Em) were found to be significantly correlating with the serum ferritin with abnormality being detected in values >5000 ng/ml and normal values <2500 ng/ml, but single correlating cutoff value was lacking which could have provided a diagnostic value. These studies, however, were based on well-chelated patients. Fernandes *et al*. recently reported a single patient with cardiac iron overload at the age of 7 years, but this study was limited to only 23 patients with TM and other forms of anemia [21].

In a case–control study conducted in Mumbai in 2008–2009, 60 cases were evaluated for cardiac iron overload toxicity through cardiac magnetic resonance imaging (MRI), in which the mean value of serum ferritin was found to be 3528.6±1958.6 ng/ml and it had significant difference in the mean cardiac MRI T2* values (23.45±13.4 ms) when compared with the controls (32.67±2.68 ms) with $p<0.01$ [22]. In the present study, we have used echocardiographic parameters such as LVEDD, EF, and LVESD and also measured serum ferritin levels in 105 β-thalassemia major children. All TM kids with serum ferritin >3000 ng/ml (cutoff value being 3261 ng/ml for EF and LVESD and 4640 ng/ml for LVEDD) should be investigated with echocardiography to screen for cardiac dysfunction due to iron overload and toxicity.

We note that again LVEDD has the highest PPV along with a good NPV. It signifies that it has got a good and reliable testing power in establishing the pre-clinical cardiac dysfunction due to iron overload based on the strength of serum ferritin values as a guide. The parameters in this regard were ordered as: LVEDD >> EF > LVESD with the Sn of 90%, 76%, and 70%, respectively. Serum ferritin is a reliable screening tool to detect cardiac iron overload through the echo parameters well before the clinical features have appeared.

This study has also some limitations. First of all, we have conducted a Phase II study, where we have only found the association but causality of the diagnostic accuracy could not be assessed. Biases and confounding factor were adjusted as far as possible but could not be avoided fully due to the study design *per se*. Hence, a follow-up study would be a better option, where we can assess a definite causality for diagnostic accuracy. Second, the less sample size of our study is another pitfall. Hence, in the future, a diagnostic study of Phase III and/or more should be done to reach a definite diagnostic accuracy.

CONCLUSION

This study is one of its kinds as it ensures a cutoff value for serum ferritin for diagnosing pre-clinical cardiac dysfunction due to iron overload through specific reliable echo parameters. Hence, health-care personnel can easily scrutinize for cardiac dysfunction, well before the clinical features of cardiac iron overload appears and hence, it helps to control and prevent morbidity and mortality, early. It has a role in improving survival rates by decreasing the morbidity and mortality due to iron overload toxicity beta-thalassemic children.

ACKNOWLEDGMENT

We are thankful to our HOD whose immense help made this manuscript possible and our colleagues who always gave us moral support and at last but not the least, the patients without whom this research would not have been possible.

REFERENCES

1. Olivieri NF. The beta-thalassemias. N Engl J Med 1999;341:99-109.
2. Modell B, Khan M, Darlison M. Survival in beta-thalassaemia major in the UK: Data from the UK thalassaemia register. Lancet 2000;355:2051-2.
3. Li CK, Luk CW, Ling SC, Chik KW, Yuen HL, Li CK, *et al.* Morbidity and mortality patterns of thalassaemia major patients in hong kong: Retrospective study. Hong Kong Med J 2002;8:255-60.
4. Rund D, Rachmilewitz E. β-Thalassemia. N Engl J Med 2005;353:1135-46.
5. Wood JC, Otto-Duessel M, Aguilar M, Nick H, Nelson MD, Coates TD, *et al.* Cardiac iron determines cardiac T2*, T2, and T1 in the gerbil model of iron cardiomyopathy. Circulation 2005;112:535-43.
6. Majd Z, Haghpanah S, Ajami GH, Matin S, Namazi H, Bardestani M, *et al.* Serum ferritin levels correlation with heart and liver MRI and LIC in patients with transfusion-dependent thalassemia. Iran Red Crescent Med J 2015;17:e24959.
7. Debaun M, Frei-jones M, Vichinsky E. Thalassemia syndromes. In: Kliegman RM, Stanton BF, St Geme JW, Schor NF, Behrman RE, editor. Nelson Textbook of Paediatrics. 20th ed. New Delhi: Reed Elsevier India Pvt. Ltd.; 2016. p. 2351.
8. Park MK. Noninvasive Techniques. In: Park MK, editor. Pediatric Cardiology for Practitioners. 6th ed. New Delhi: Elsevier India Pvt. Ltd.; 2014. p. 87.
9. Park MK. Noninvasive Techniques. In: Park MK, editor. Pediatric Cardiology for Practitioners. 6th ed. New Delhi: Elsevier India Pvt. Ltd.; 2014. p. 584, 602.
10. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, *et al.* Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001;22:2171-9.
11. Piperno A. Classification and diagnosis of iron overload. Haematologica 1998;83:447-55.
12. Aldouri MA, Wonke B, Hoffbrand AV, Flynn DM, Ward SE, Agnew JE, *et al.* High incidence of cardiomyopathy in beta-thalassaemia patients receiving regular transfusion and iron chelation: Reversal by intensified chelation. Acta Haematol 1990;84:113-7.
13. Tanner MA, Galanello R, Dessi C, Smith GC, Westwood MA, Agus A, *et al.* Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. J Cardiovasc Magn Reson 2008;10:12.
14. Engle MA, Erlandson M, Smith CH. Late cardiac complications of chronic, severe, refractory anemia with hemochromatosis. Circulation 1964;30:698-705.
15. Yang G, Liu R, Peng P, Long L, Zhang X, Yang W, *et al.* How early can myocardial iron overload occur in beta thalassemia major? PLoS One 2014;9:e85379.
16. Wood JC. Cardiac iron across different transfusion-dependent diseases. Blood Rev 2008;22 Suppl 2:S14-21.
17. Shivanna NH, Murthy GR, Ambica, Munirathnam G. Cardiac abnormalities in children with thalassemia major correlation of echocardiographic parameters with serum ferritin levels. Int J Contemp Pediatr 2016;3:12-5.
18. Tziomalos K, Perifanis V. Liver iron content determination by magnetic resonance imaging. World J Gastroenterol 2010;16:1587-97.
19. Olivieri NF, Brittenham GM, Matsui D, Berkovitch M, Blendis LM, Cameron RG, *et al.* Iron-chelation therapy with oral deferiprone in patients with thalassemia major. N Engl J Med 1995;332:918-22.
20. Wood JC, Tyszka JM, Carson S, Nelson MD, Coates TD. Myocardial iron loading in transfusion-dependent thalassemia and sickle cell disease. Blood 2004;103:1934-6.
21. Silvilairat S, Sittiwangkul R, Pongprot Y, Charoenkwan P, Phornphutkul C. Tissue doppler echocardiography reliably reflects severity of iron overload in pediatric patients with beta thalassemia. Eur J Echocardiogr 2008;9:368-72.
22. Merchant R, Joshi A, Ahmed J, Krishnan P, Jankharia B. Evaluation of cardiac iron load by cardiac magnetic resonance in thalassemia. Indian Pediatr 2011;48:697-701.

Funding: None; Conflict of Interest: None Stated.

How to cite this article: Majhi SC, Mishra NR, Panda PC, Biswal SS. Serum ferritin as a diagnostic marker for cardiac iron overload among beta-thalassemia major children. Indian J Child Health. 2019; 6(6):269-272.

Doi: 10.32677/IJCH.2019.v06.i06.003