Original Article

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Quick Response Code:

Website: www.ajts.org
DOI: 10.4103/ajts.AJTS_3_17

Relationship between T2* magnetic resonance imaging-derived liver and heart iron content and serum ferritin levels in transfusion-dependent thalassemic children

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Abstract:

CONTEXT: T2* magnetic resonance imaging (MRI) is being increasingly used for the assessment of organ iron content in thalassemics, but cost is a major prohibitive factor for repeated measurements. If serum ferritin correlates well with the T2* MRI liver and heart, it will be economical and more simple tool to assess organ iron deposition.

AIMS: The aim of this study was to find out the relationship between serum ferritin level and T2* MRI-derived liver and heart iron content in transfusion-dependent thalassemic children

SETTINGS: Thalassemia day-care center of a teaching hospital

DESIGN: This was a cross-sectional study

SUBJECTS AND METHODS: Seventy-three transfusion-dependent beta thalassemic children belonging to 2–18 years of age were subjected to T2* MRI of heart and liver to assess their iron content. Values obtained here were related to serum ferritin.

STATISTICAL ANALYSIS USED: Keeping the correlation between serum ferritin and T2* MRI as primary outcome, spearman's correlation coefficient was calculated.

RESULTS: We found poor (negative) correlation between serum ferritin level and T2* MRI liver (r = -0.448, P = 0.000) but no correlation between serum ferritin and T2*MRI heart (r = -0.221, P = 0.060).

CONCLUSIONS: Serum ferritin cannot reliably predict the liver and heart iron content in Indian children with β thalassemia.

Keywords:

Hemolytic anemia, iron deposition, organ magnetic resonance imaging

Introduction

Transfusion dependency in β-thalassemia major, in association with enhanced iron absorption, ineffective erythropoiesis, and peripheral hemolysis lead to iron deposition in various organs including liver, heart, and endocrine organs.[1] Once cell's iron handling capacity is exhausted, level of labile iron pool (LIP) starts increasing.[2] LIP-induced liver and heart dysfunction are the major cause of morbidity and mortality in these children.[1,3]

Several serum markers: ferritin, iron, total iron binding capacity, and transferrin saturation are commonly used to assess iron status. Among these, serum ferritin is the

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Submission: 07-01-2017
Accepted: 29-04-2017

How to cite this article: Suthar K, Goyal VK, Sharma P, Deopa B, Rathore PS, Bishnoi RK. Relationship between T2* magnetic resonance imaging-derived liver and heart iron content and serum ferritin levels in transfusion-dependent thalassemic children. Asian J Transfus Sci 2018;12:69-74.

most frequently used as it is inexpensive, convenient, and is widely available. However, serum ferritin may not truly reflect the organ iron overload as it represents only 1% of the total iron pool. Besides this, being an acute-phase reactant, ferritin can be nonspecifically raised in any inflammatory condition, which further reduces its utility in monitoring iron status of body.[3-5]

Liver biopsy with measurement of iron concentration by atomic absorption spectroscopy was once considered to be the gold standard for quantitative assessment of live iron content. However, patchy involvement of liver and excessive fibrosis may underestimate the true iron status.[6] Moreover, it is an invasive procedure and also not free of complications. Thus, it cannot be repeatedly used for this purpose. Myocardial biopsy also has the similar limitations.[7]

Recently, magnetic resonance imaging (MRI) has been increasingly used to assess the organ iron content. R2 MRI and R2* MRI liver have shown a good correlation with liver biopsy.[5,8] Similarly, T2* MRI heart has shown a good correlation with myocardial biopsy and left ventricular ejection fraction.[9,10] MRI being noninvasive and safe can be used repeatedly, and it also allows quantification of iron in both liver and heart at the same time. Now, iron chelation therapy is guided by organ iron deposition and MRI is considered the gold standard for this purpose.[11]

However, nonavailability of MRI at each center is a major issue in developing countries, where still serum ferritin is preferred for monitoring iron load. Cost is also a prohibitive factor for repeated MRI examination.[12] Optimum use of serum ferritin supplemented by MRI-based assessment is required in resource-limited settings. Few studies have correlated serum ferritin with T2* MRI heart and liver but with variable results in different ethnic groups. Most of these studies have been done on a heterogeneous population which includes mainly adults and few children. Exclusive pediatric data in Indian population are lacking. This study was planned to evaluate the role of serum ferritin, considering T2* MRI as a gold standard for assessment of iron deposition in liver and heart in Indian children with β thalassemia major.

Our primary objective was to find out the correlation between serum ferritin level and T2* MRI liver and heart in this population and to derive a serum ferritin cutoff level to predict abnormal T2* MRI.

Subjects and Methods

This cross-sectional descriptive study was conducted over a period of 6 months at thalassemia day-care center of a teaching hospital. The Ethical Board of the Institute approved the study. All thalassemic children attending this day-care center are registered here. Most of the children visit every fortnightly for blood transfusion. This center also maintains the clinical and demographic details of all the registered children. From this registry of 158 patients, 79 (as per sample size calculated) transfusion-dependent children ≥2 years of age were randomly (bearing odd registration number) selected. All required details were drawn from the same registry. Parents of all enrolled patients were informed about the study, and those who consented were included in the study. Tests for hepatitis B surface antigen (HBsAg) by chromatographic immunoassay (AccuTest HBsAg), IgM antibody for hepatitis C virus (HCV-TRI-DOT), and antibodies to HIV by rapid card test (QUADRO HIV 1–2 Ab) were repeated to know the current status. Serum levels of aspartate transaminase and alanine transaminase were measured, and all those whose enzymes were elevated to more than 2.5 times of upper limit of normal values (>100 units/L) were excluded from the study.

Nonfasting serum ferritin level was measured by chemiluminescence immunoassay, USA. Active infection was ruled out clinically at the time of blood collection for ferritin. One-point five tesla MRI (Philips Achieva, the Netherlands) was used to collect T2* values. Surface coil was used to broadcast and to receive the radiofrequency signals. Respiratory and heart movements were monitored using respiratory sensor and electrocardiography and images in deep inspiration were auto clicked. Motion artifacts were suppressed by spatial presaturation slabs. For assessment of liver, a homogeneous region of interest (ROI) was outlined in the liver parenchyma, and for heart, a homogeneous full-thickness ROI was chosen in the ventricular septum. Gradient echo sequence was used to scan liver (L) and heart (H); slice thickness 10 mm (L) and 8 mm (H), echo time 16 (200 ms) for L and 6 (200 ms) for H, repetition time 1000 ms (L, H), flip angle 90° (L, H), base resolution matrix 512 pixels (L, H), field of view 35 cm × 10 cm (L) and 10 cm × 15 cm (H), and sampling bandwidth - 125 k Hz (L, H).

The mean signal intensity of region was measured for each image and plotted against the echo time. Formulas derived by Hankins *et al*.[13] and Carpenter *et al*.[14] were used to assess iron content in liver and heart respectively. T2* values obtained on MRI were defined as follows: for liver (normal >11.4, abnormal ≤11.4 ms) and for heart (normal >20, abnormal ≤20 ms).

Statistics

All statistical analyses were performed using SPSS Software Version 21 (IBM, Armonk, New York,

USA). For all statistical purposes, $P < 0.05$ was considered statistically significant. Shapiro–Wilk test was performed to analyze the distribution of data; gaussian versus nongaussian. As all of our variables followed nongaussian distribution, nonparametric tests were applied for analysis. Spearman correlation coefficient (r) was graded[15] as no correlation - 0–0.25, poor - 0.25–0.50, moderate to good - 0.5–0.75, very good to excellent - 0.75–1. Coefficient of determination (r^2) was calculated to express the strength of correlation. To compare median ferritin values between normal and abnormal T2* MRI groups, Manny–Whitney test was applied. Receiver operating characteristic curve (ROC) analysis was done to determine the optimum serum ferritin level to predict abnormal MRI values (iron excess in liver and heart). Keeping α error (two-tailed) 0.05, power of study 99% (β error - 0.01), and expecting moderate correlation (correlation coefficient 0.5), sample size was calculated to be 64.

Results

From the registry of 158 thalassemic children fulfilling our criteria, 79 were enrolled (odd numbers included). Of them, four did not turn up and two had elevated liver enzymes, so they were excluded from the study. Of 73 included children, 48 (65.75%) were males and 25 (34.25%) were females. Five (6.85%) were positive for HBsAg, 28 (38.36%) for hepatitis C, and one (1.37%) was positive for HIV. Descriptive statistics of all observed parameters have been summarized in Table 1.

A negative but poor correlation was observed between serum ferritin and T2* MRI liver ($r = –0.448$, $P = 0.000$) with 0.201 strength of correlation. No correlation was found between serum ferritin and T2* MRI heart ($r = –0.221$, $P = 0.060$). T2* MRI liver also did not correlate with that of heart ($r = 0.176$, $P = 0.137$) [Table 2].

T2* MRI was abnormal in 66/73 (90.41%) children for liver, but for heart, it was abnormal in only 14 (19.17%) thalassemics. Median serum ferritin level in abnormal T2* MRI liver group was higher than the normal (1848.00 vs. 831.00 ng/ml, $P = 0.000$), similar findings were observed with the heart also (2727.500 vs. 1367.00 ng/ml, $P = 0.004$) [Figures 1 and 2]. ROC curve plotted to determine optimum serum ferritin level to predict abnormal T2* MRI, showed excellent discrimination (AUC - 0.904, $P < 0.0001$) for liver and good discrimination for heart (AUC - 0.749, $P = 0.0014$). Serum ferritin level cutoffs were calculated to be >1100 and >1619 ng/ml with sensitivity 77.3% and 85.7%, specificity 100% and 61%, positive predictive value 100% and 34.3%, negative predictive value 31.8% and 94.7%, and Youden index J - 0.772 and 0.467 for liver and heart, respectively.

Discussion

As meta-analysis of previous studies [Table 3, total sample size 716] showed total (fixed effects) $r = –0.622$ (95% confidence interval = –0.575– –0.666), we selected r value to be 0.5 (at least moderate correlation) for our study to correlate serum ferritin and T2* MRI liver, but the present study showed poor negative correlation ($r = –0.448$). At this value, still the power of study remains >90% as we had included a total of 73 children. We could not establish the correlation between serum ferritin level and T2* MRI heart ($r = 0.221$, $P = 0.060$). We did not find any correlation between T2* MRI liver and heart (0.176, $P = 0.137$). After compressing the continuous outcome variable into binomial data, normal and abnormal, we derived that serum ferritin level >1100 ng/ml can predict abnormal liver iron deposition (AUC - 0.904, Youden index J - 0.7727, $P < 0.0001$). Similarly, for abnormal heart, serum ferritin value came out to be >1619 ng/ml but

Table 1: Clinical and paraclinical characteristics of the study cohort

Parameters	Range	Mean±SD	Median (IQR)†	P‡
Age (years)	2-18	9.062±4.685	8 (5-12)	0.002
Weight (kg)	12-61	24.918±10.512	23 (16-31)	0.000
Serum AST (U/L)	20-100	59.014±23.303	54 (39-80)	0.002
Serum ALT (U/L)	20-100	54.15 1±23.463	45 (37-70)	0.000
Serum ferritin level (ng/ml)	210-5033	1895.068±1126.443	1563 (991-2475)	0.0001
T2* MRI liver (ms)	0.900-39.200	5.604±5.891	4.400 (3.200-5.300)	<0.0001
T2* MRI heart (ms)	3.800-66.200	30.281±12.684	33.00 (26.300-38.400)	0.001

†IQR (25th-75th centile), ‡Shapiro Wilk test. AST = Aspartate transaminase, ALT = Alanine transaminase, MRI = Magnetic resonance imaging, SD = Standard deviation, IQR = Inter quartile range

Table 2: Correlation between serum ferritin levels and magnetic resonance imaging values

Input variable	Output variable	Spearman correlation matrix (rs)	Coefficient of determination	P
Serum ferritin level	T2*MRI liver	–0.448	0.201	0.000
	T2*MRI heart	–0.221	0.049	0.060
T2*MRI liver	T2* MRI heart	0.176	0.031	0.137

MRI = Magnetic resonance imaging

Table 3: Comparison of correlation coefficient

Author, publication year	Sample size	Correlation coefficient *r* (*P*)	
		Serum ferritin versus T2* MRI liver	Serum ferritin versus T2* MRI heart
Majd *et al*., 2015[16]	85	−0.698 (0.001)	−0.329 (0.002)
Fahmy *et al*., 2015[17]	70	−0.318 (0.007)	−0.077 (0.528)
Eghbali *et al*., 2014[18]	60	−0.297 (0.021)	−0.120 (0.361)
Azarkeivan *et al*., 2013[19]	156	−0.535 (<0.001)	−0.361 (<0.001)
Kolnagou *et al*., 2013[20]	20	−0.63	No correlation
Fragasso *et al*., 2011[21]	99		−0.19 (0.057)
Zamani *et al*., 2011[22]	210	−0.586 (0.000)	
Assis *et al*., 2011[23]	115	−0.878 (0.001)	
Anderson *et al*., 2001[10]	30		0.10 (0.32)

MRI = Magnetic resonance imaging

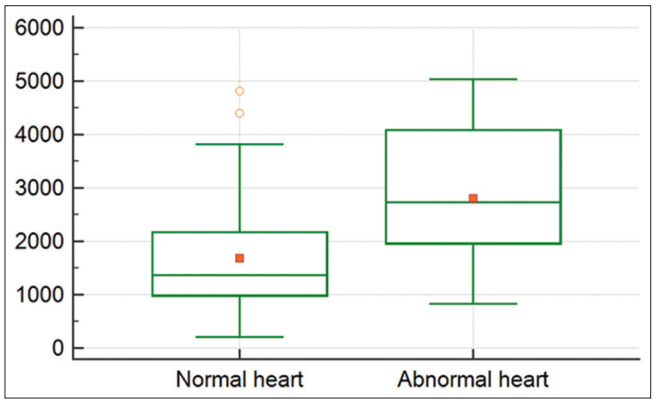

Figure 1: Box and whisker graph comparing serum ferritin level in normal and abnormal heart (T2* MRI). y axis - serum ferritin level (ng/ml)

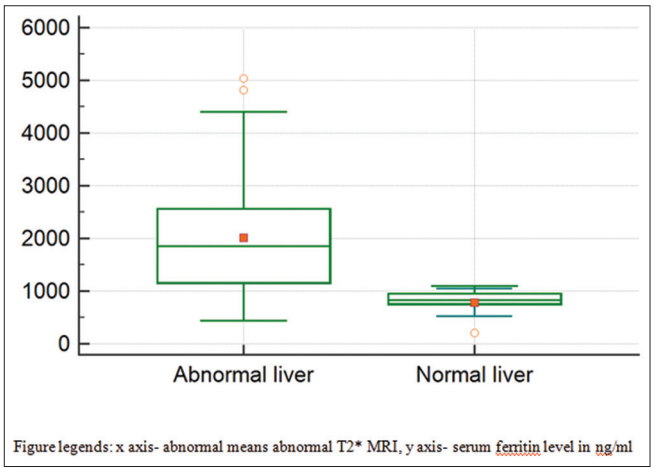

Figure 2: Box and whisker graph comparing serum ferritin level in normal and abnormal liver (T2* MRI). x axis - abnormal means abnormal T2*MRI, y axis - serum ferritin level (ng/ml)

with a low discrimination (AUC - 0.749, Youden index J - 0.4673, *P* = 0.0014).

Limitations of our study included single measurement of ferritin which was done within 3 days of MRI examination. An average value of the last 6 months ferritin level might have reflected the true status, but some of the recent studies also have used the single measurement of serum ferritin.[19] Clinical criteria alone were used to rule out active infection/inflammation, without use of any objective parameter such as C-reactive protein estimation. This can be taken as the second limitation of our study.

Similar to our findings, poor negative correlation between serum ferritin level and T2* MRI liver was observed by Eghbali *et al*.[18] and Fahmy *et al*.[17] also. On the other hand, Azarkeivan *et al*.,[19] Kolnagou *et al*.,[20] and Zamani *et al*.[22] showed moderate correlation, and in contrast to our study, Assis *et al*.[23] demonstrated excellent correlation between serum ferritin and T2* MRI liver.

Similar to our study, most of the previous studies[10,17,18,20,21] also failed to correlate serum ferritin level and T2* MRI heart (either *r* was <0.25 or *P* value was >0.05) except the study by Majd *et al*.,[16] who showed poor negative correlation (*r* = −0.329, *P* = 0.002) between these two.

Previously, it was thought that liver being the largest storage site of iron if overloaded will proportionately affect the other organs such as heart also. However, we failed to correlate these two parameters and our results are consistent with the findings observed by Kolnagou *et al*.,[20] Assis *et al*.[23] (both no correlation), and Anderson *et al*.[10] (*r* = 0·15, *P* = 0·11). In contrast, Azarkeivan *et al*.[19] and Christoforidis *et al*.[24] were able to demonstrate the poor correlation (*r* = 0.281, *r* = 0.42, respectively) between these two.

These variable results could be because of differences in clinical, genetic, and demographic characteristics of study population such as age, sample size, serum ferritin levels, chelating protocols, and iron kinetics of different organs. Our study population was more homogenous with narrow age range (2–18 years) in comparison to previous studies[19,20,22,23] where age group ranged from as young as 5 years to as old as 54 years. Overall, our population was also quite younger than previous studies; mean age - 9.06 ± 4.64 years in our study versus 22.79 ± 7.1 and 22.77 ± 6.2 thalassemia major and intermedia groups, respectively, in Majd *et al*.,[16]

21.25 years in Assis *et al*.,[23] 24.1 ± 5.4 years in Azarkeivan *et al*.,[19] and 25.59 ± 8.5 years in Zamani *et al*.[22] In all these studies, sample size also fluctuated from as small as 20 in Kolnagou *et al*.[20] to as large as 210 in Zamani *et al*.[22]

Exclusion criteria were also not fixed. We excluded only advanced liver dysfunction patients. Fahmy *et al*.[17] excluded liver diseases as well as hepatitis B or C positive also. Eghbali *et al*.[18] excluded not only these but advanced cardiac disorder parients also. In the present cohort, 6.85% children were positive for HBsAg and 38.36% for hepatitis C. We did not exclude these as previous study has shown that HCV positivity do not affect the relation between ferritin and T2* MRI liver or heart.[19]

Mean serum ferritin level also varied from 1600 ± 1805 to 2676.5 ± 2051.7 ng/ml in previous studies.[23,21] Azarkeivan *et al*.[19] noted that serum ferritin level more than 4000 ng/ml weakens the correlation between serum ferritin and T2* MRI (liver and heart), the similar finding was observed by Worwood *et al*.[25] also. They suggested that maximum rate of synthesis and release of glycosylated ferritin by reticoendothelial cells, once fully saturated may be responsible for this phenomenon.

Timing of sample collection for ferritin was again unfixed. Eghbali *et al*.[18] took a mean of last 3 months serum ferritin levels. Fragasso *et al*.,[21] though correlated with only cardiac MRI, took a mean of previous 12 months by measuring serum ferritin every 2 month. Furthermore, there was a large geographic variation in study population. Most of the previous studies have been done either in Middle East[16-19,22] or Europe.[10,20,21] Our study population belonged to South Asia.

As involvement of heart and liver is the major determinants of mortality in thalassemia, these organs need to be screened regularly for iron deposition during chelation therapy. Now, MRI-based organ assessment is considered the gold standard, but the machine is not widely available and cost is also a prohibitive factor in developing regions. Serum ferritin is a cheaper option, but our results do not favor its use for continuous monitoring, especially for heart. In these settings, serum ferritin values can be used as an alarm for need of MRI-based assessment.

Conclusion

Although serum ferritin cannot reliably predict the liver and heart iron content in Indian children with β thalassemia, in resource-limited settings, initially serum ferritin can be monitored, and once the values exceed 1100 ng/ml and 1619 ng/ml, MRI-based assessment of liver and heart should be done, respectively.

Acknowledgments

We would like to thank Dr. Hemant Jangid (MD Radiodiagnosis) for magnetic resonance imaging reporting.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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