ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 15, Issue, 04, pp.24225-24229, April, 2023
DOI: https://doi.org/10.24941/ijcr.45041.04.2023

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SERUM FERRITIN LEVELS IN COMPARISON WITH MRI IN THE CLINICAL MANAGEMENT OF β-THALASSEMIA MAJOR

Saroja Kondaveeti[1], Padma Gunda[1], Mamata Manne[1], Sundaresh Peri[2], Ravi Kumar Allum[1] and Suman Jain[1,*]

[1]Department of Molecular Genetics, Kamala Hospital and Research Centre for Thalassemia and Sickle Cell Patients-A unit of Thalassemia and Sickle Cell Society, Hyderabad, India
[2] Statistician Cum Lecturer in Statistics (Retired), Department of Community Medicine, Kakatiya Medical College, Warangal, India

ARTICLE INFO

Article History:
Received 14th January, 2023
Received in revised form
17th February, 2023
Accepted 10th March, 2023
Published online 18th April, 2023

Key words:

Beta Thalassemia Major, Iron Overload, Serum Ferritin, Magnetic Resonance Imaging, Correlation, Chelation.

**Corresponding Author:*
Suman Jain

ABSTRACT

Background: Iron overload is a serious complication in patients with beta thalassemia major (β-TM) resulting in cardiomyopathy, liver cirrhosis and arrhythmias. Assessing iron overload regularly in these patients is imperative for initiating effective chelation therapy. Serum ferritin measurements and Magnetic Resonance Imaging (MRI) offer reliable methods for evaluating iron overload. **Objective:** In the present study, iron overload was assessed by serum ferritin levels and MRI and the findings were correlated in order to propose serum ferritin as an easy and inexpensive method for evaluating iron overload in thalassemia patients. **Methods:** A total of 100 β-TM patients aged 8 to 30 years of both sexes who were on regular blood transfusions and chelation therapy were evaluated for various demographic parameters. Complete blood count (CBC) measurements were done using cell counter. Serum ferritin levels were determined using chemiluminescence method and MRI. **Results:** A moderate negative correlation (r=-0.6037) was observed between the average serum ferritin and T2* Liver and was statistically significant (P<0.0001) which implies that as the average ferritin recorded for thalassemia patients increases, the T2* MRI value reduces which means that severity of iron overload in the liver increases. A weak negative correlation (r=-0.3342) was also seen for average ferritin levels and T2* MRI Heart which was also statistically significant (P=0.0007). **Conclusion:** A significant negative correlation between serum ferritin levels and MRI T2*liver and heart suggests that serum ferritin levels can be used to diagnose iron over load in patients with thalassemia as an alternative where T2* MRI is not available.

Citation: ***Saroja Kondaveeti, Padma Gunda, Mamata Manne, Sundaresh Peri, Ravi Kumar Allum and Suman Jain.*** **2023.** "Serum Ferritin Levels in comparison with MRI in the clinical management of β-Thalassemia Major*". International Journal of Current Research*, 15, (04), 24225-24229.

INTRODUCTION

β-thalassemia major (β-TM) is a hereditary blood disorder caused by deficient synthesis of the β-globin chains leading to ineffective erythropoiesis, hemolysis and thereby, marked anemia. It represents a significant health problem with more than 400,000 new-borns affected every year worldwide.[1] It is predicted that in the next 20 years, about 900,000 patients with thalassemia will be born in the world, with 95% of them being in Asia especially in India and Middle East.[2] One of the major complications of β-thalassemia is iron overload which results due to regular blood transfusions given to patients to maintain the hemoglobin levels in order to sustain their life. Iron overload is so common in patients that it has been referred to as "second disease" during the treatment of the first[3] and results in a number of other diseases and serious clinical complications like cardiomyopathy, arrhythmias and liver cirrhosis due to iron deposits in the organs.

Iron is stored mostly in the liver in the form of ferritin. Ferritin is a protein with a capacity of about 4500 iron (III) ions per protein molecule. In the body, small amounts of this ferritin are secreted into the plasma (or serum) whose concentration is positively correlated with the size of the total body iron stores in the absence of inflammation. When the capacity for storage of iron in ferritin exceeds, a complex of iron with phosphate and hydroxide forms called as hemosiderin. In the event of iron overload, this excess hemosiderin gets deposited in the liver, heart and other organs leading to their failure and leading to death. Hence, an accurate assessment of the body iron is essential for managing chelation therapy in patients with β-thalassemia major who receive multiple transfusions. Further, cardiomyopathy resulting from iron overload is potentially reversible, even in advanced stages, if vigorous chelation therapy is applied.[4] Therefore, early intensification of iron chelation therapy by a suitable technique could reduce mortality from this reversible cardiomyopathy.[5]

Iron overload can be assessed by indirect measurements, such as the levels of serum ferritin used to assess body iron stores as well as direct measurements of iron deposition in the liver and heart using Magnetic Resonance Imaging (MRI) techniques. Studies have been conducted on different aspects of using MRI to determine the patients' iron overload and its association with serum ferritin level[6-9]but the results remain controversial. Though MRI offers a selective, noninvasive, and safe method[10-11]for determining the body's iron level in β-thalassemia patients, it is expensive and not an easy examination in children. Hence, the present study aims to evaluate iron overload in β-thalassemia patients using MRI and correlate it with serum ferritin levels so that if a positive correlation is found then serum ferritin can be used to assess the iron levels in patients easily and inexpensively.

MATERIALS AND METHODS

Study design: For the present study a total of 100 β-thalassemia major patients aged 8 years to 35 years (upper age limit based on our demographic profile of patients) who were on regular blood transfusions and chelation therapy were recruited from among the patients diagnosed and registered at Thalassemia and Sickle Society, Hyderabad, India. Written informed consent was taken from all the patients willing to participate in the study. The study was approved by institutional ethical committee. These patients receive blood transfusions every three to four weeks with an average amount of received packed cell of 150-180 ml/kg.

Diagnosis: The patients were diagnosed by the clinicians based on low hemoglobin level, low mean corpuscular volume (MCV), low mean corpuscular hemoglobin (MCH), and increased HbF (fetal hemoglobin) levels as measured by high performance liquid chromatography (HPLC).

Inclusion and Exclusion Criteria

Inclusion Criteria: Only patients with β-thalassemia major aged 8 years to 35 years (upper age limit based on our demographic profile of patients), of both sexes undergoing regular blood transfusions and iron chelating therapy, were included in the study.

Exclusion Criteria: Patients with severe heart failure and congenital cardiac anomalies; patients who had been undergoing iron chelating therapy for less than 6 months, and those unwilling to comply with the research protocol were excluded from the study.

Sample size: Sample size calculation: Considering $\alpha = 0.05$, power = 90% and correlation between serum ferritin levels and T2* MRI of liver: $r = -0.44$[12], 100 patients with β-thalassemia major were considered.

Collection of Blood Sample: For performing of laboratory tests, before each blood transfusion, 2 ml EDTA-anticoagulated blood for Complete Blood Count and 3ml non-anticoagulated blood for serum ferritin measurement were collected from β-thalassemia major patients who were willing to participate in the study after explaining to them the importance and outcome of the study. CBC measurements were carried out by cell counter.

Estimation of Serum Ferritin Levels: The Serum ferritin levels were determined using chemiluminescence method in Neo Lumax as per the manufacturer's protocol. The mean of serum ferritin level was recorded for every 3 months during one year and the average ferritin values were correlated with MRI findings.

Assessment of Iron overload in the liver and heart: MRI T2* was used to determine the patients' cardiac and liver iron load using the MRI device. In MRI T2* relaxation time shows the severity of iron overload in the heart and liver. Echocardiography was performed for all participants and ejection fraction (EF) - reference range 55–75 % - was calculated.

MRI-T2* (through commercial source) was carried out to determine iron burden in liver and heart. For liver MRI, a single breath-hold technique using a multi-echo gradient echo (8–16 echoes) sequence and for heart a single midpapillary ventricular short-axis slice using a cardiac-gated, segmented and multi-echo gradient echo (8 echo) sequence will be obtained. In the heart MRI-T2*, conduction time more than 20-ms will be considered as Normal iron load, between 15 – 20-ms as Mild, 10–15 ms as Moderate and less than 10 ms as an indicative of Severe myocardial siderosis. In the liver MRI, iron load will also be classified as: Normal (T2* more than 15.4ms), Mild (T2* between 4.5-15.4ms), Moderate (T2* between 2.1-4.5ms) and Severe (T2* ≤ 2.1ms). The findings of MRI and Serum Ferritin was assessed concomitantly.

Data collection & statistical analysis

Data collected for the study

Collection of data: From each patient information was collected using a proforma which included details on the following:

#	Variable name	Description
1	S.No	Serial Number
2	Patient	Patient name
3	Card No	Patient Case sheet number
4	Age (yrs)	Age of patient in Years
5	Sex	Sex of the patient: Female/Male
6	Ferritin 1 (Jan-21)	Ferritin-1 (Jan-21) taken during the month
7	Ferritin 2 (Apr-21)	Ferritin 2 (Apr-21)
8	Ferritin 3 (Jul-21)	Ferritin 3 (Jul-21)
9	Ferritin 4 (Oct-21)	Ferritin 4 (Oct-21)
10	Avg.Ferritin	Average Ferritin (Average of the above 4 values)
11	SD-Ferritin	Standard Deviation of the 4 Ferritin values
12	Liver T2* millisecs (Oct-21)	Liver T2* millisecs (Oct-21)
13	Liver Severity	Liver Severity (Normal, Low, Medium, Severe)
14	Heart T2* millisecs	Heart T2* millisecs
15	Heart Severity	Heart Severity (Normal, Low, Medium, Severe)
16	Total Bilirubin mg/dl (Oct-21)	Total Bilirubin mg/dl (Oct-21)
17	Indirect mg/dl	Indirect mg/dl
18	SGPT U/L	SGPT U/L
19	SGOT U/L	SGOT U/L
20	Alkaline Phosphatase IU/L	Alkaline Phosphatase IU/L
21	Albumin gms/dl	Albumin gms/dl
22	No. of Transfusions	Total Number. of Transfusions
23	Ejection Fraction	Ejection Fraction
24	PAH	PAH
25	LV filling	LV filling
26	DNA Mutation	DNA Mutation
27	DOFT	Date of first Transfusion
28	DOLT	Date of latest Transfusion
29	Days In TRNF	Days between first and latest Transfusions (DOLT-DOFT)
30	No of Trf per year	Average Num of Transfusions per year (R22/R29*365)

Data Analysis: Data was analyzed by SPSS software (IBM SPSS Statistics version 20.0). Descriptive data were presented as mean, standard deviation and boxplots. Kruskal-Wallis test was used for the comparison among three or more groups of patients. Correlation analysis between serum ferritin levels and MRI variables was done using the Pearson's test and $p < 0.05$ was considered to be significant.

RESULTS

A total of 100 thalassemia patients, 56 (56%) males and 44 (44%) females were enrolled in the present study.

Table 1. Comparison of mean serum ferritin levels among the four groups of thalassemia patients based on T2* Liver

Liver Severity	n	Avg. Ferritin	SD	*ANOVA; **Kruskal Wallis test
Normal	14	731	211	Both parametric and Non-parametric tests show P<0.001
Mild	29	1382	508	
Moderate	39	3360	1763	
Severe	18	4246	1357	
Total	100	2578	1799	

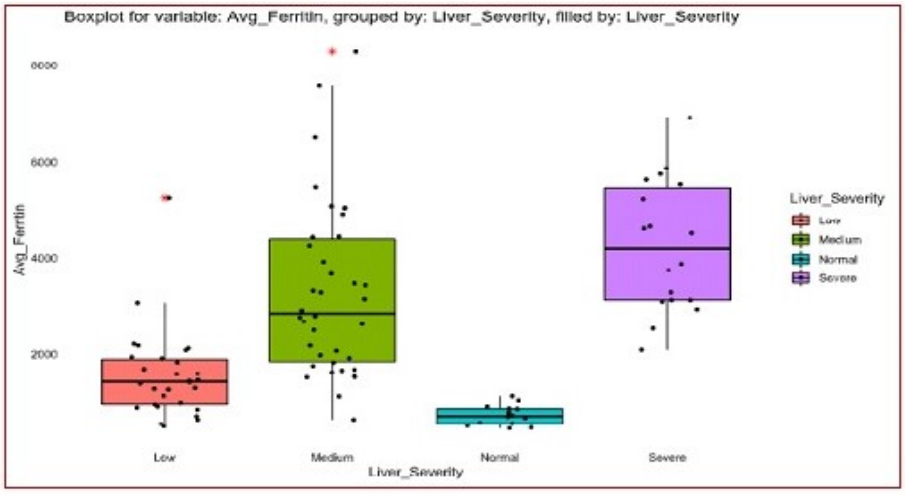
Table 2. Pearson Correlation Matrix of the study Variables

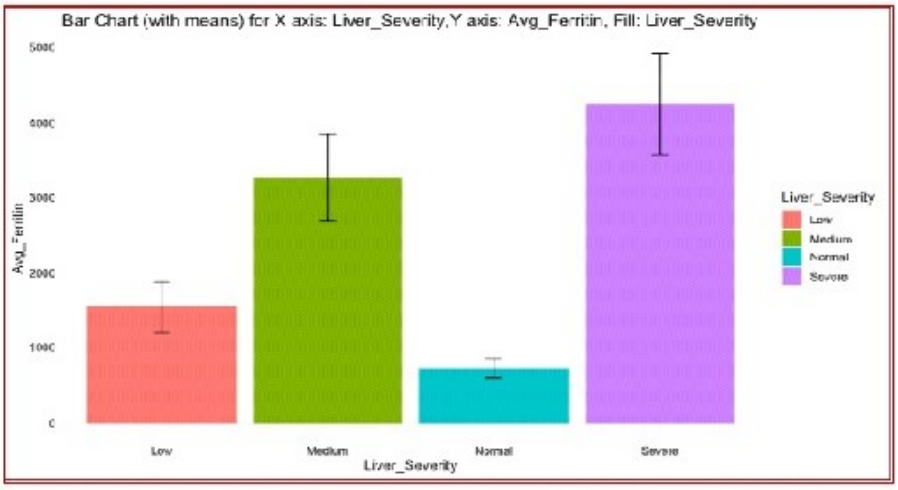
Variables	Statistics	Age Yrs	Avg Ferritin	Liver T2 millisecs	Heart T2 millisecs	Total Bilirubin mg dl	Indirect mg dl	SGPT U L	SGOT U L	Alkaline Phosphatase IU L
Age.Yrs.	r	1	0.0966	0.0022	-0.1406	0.2816	0.2558	-0.0597	-0.0469	-0.3727
	p		0.3388	0.9828	0.1631	0.0045	0.0102	0.5555	0.6434	0.0001
	n	100	100	100	100	100	100	100	100	100
Avg Ferritin	r	0.0966	1	-0.6037	-0.3342	-0.3637	-0.3499	0.6097	0.5633	-0.1019
	p	0.3388		<.0001	0.0007	0.0002	0.0004	<.0001	<.0001	0.3132
	n	100	100	100	100	100	100	100	100	100
Liver T2 millisecs	r	0.0022	-0.6037	1	0.2578	0.3001	0.2605	-0.2976	-0.3075	0.3602
	p	0.9828	<.0001		0.0096	0.0024	0.0089	0.0026	0.0019	0.0002
	n	100	100	100	100	100	100	100	100	100
Heart T2 millisecs	r	-0.1406	-0.3342	0.2578	1	0.1204	0.1304	-0.3108	-0.2964	-0.0198
	p	0.1631	0.0007	0.0096		0.2329	0.1961	0.0016	0.0027	0.8453
	n	100	100	100	100	100	100	100	100	100
Total Bilirubin mg dl	r	0.2816	-0.3637	0.3001	0.1204	1	0.9896	-0.204	-0.1738	0.071
	p	0.0045	0.0002	0.0024	0.2329		<.0001	0.0418	0.0837	0.4825
	n	100	100	100	100	100	100	100	100	100
Indirect mg dl	r	0.2558	-0.3499	0.2605	0.1304	0.9896	1	-0.2099	-0.1769	0.0607
	p	0.0102	0.0004	0.0089	0.1961	<.0001		0.0361	0.0783	0.5488
	n	100	100	100	100	100	100	100	100	100
SGPT U/L	r	-0.0597	0.6097	-0.2976	-0.3108	-0.204	-0.2099	1	0.9549	-0.0012
	p	0.5555	<.0001	0.0026	0.0016	0.0418	0.0361		<.0001	0.9902
	n	100	100	100	100	100	100	100	100	100
SGOT U/L	r	-0.0469	0.5633	-0.3075	-0.2964	-0.1738	-0.1769	0.9549	1	-0.0345
	p	0.6434	<.0001	0.0019	0.0027	0.0837	0.0783	<.0001		0.7332
	n	100	100	100	100	100	100	100	100	100
Alkaline Phosphatase IU/L	r	-0.3727	-0.1019	0.3602	-0.0198	0.071	0.0607	-0.0012	-0.0345	1
	p	0.0001	0.3132	0.0002	0.8453	0.4825	0.5488	0.9902	0.7332	
	n	100	100	100	100	100	100	100	100	100

Table 3. Comparison of mean serum ferritin levels among the four groups of thalassemia patients based on T2* Heart

Heart Severity	N	Avg. Ferritin	SD
Normal	88	2486	1788
Mild	3	3080	913
Moderate	3	3427	1968
Severe	6	3243	2296
Total	100	2578	1799

Fig 1. Box plot and Bar Chart of Ferritin values by Liver Severity





The patients mean ±SD ferritin level was 2578±1799 ngm/ml in liver.Majority (39%) of the patients showed moderate iron overload which was found to be statistically significant (P<0.001). Statistically significant difference (P<0.001) was also observed in the distribution of iron load in the four groups of thalassemia patients based on T2* liver (Table-1; Fig-1). A moderate negative correlation (r=-0.6037) was observed between the average serum ferritin and T2* Liver which was statistically significant (P<0.0001; Table-2). The relaxation time of cardiac MRI was >20msecs in 50patients, between 15-20msecs in 1 patient, between 10-15 msecs in 1 patient and <10msecs in 4 patients. Mean Iron load in heart was 2578±1799 ngms/ml with 88% of the patients showing normal iron overload and 6% of the patients having severe iron overload in the heart. There was a weak negative correlation between (r=-0.3342) average ferritin levels and T2* MRI Heart which was statistically significant (P=0.0007; Table-2 & 3). The mean iron overload in the liver was 2578±1799ngms/ml. A weak negative correlation (r=-0.2976, -0.3075) was also observed between MRI T2* liver and liver function parameters SGOT and SGPT which were statistically significant (P=0.0026, 0.0019). A positive correlation (r=0.3001, 0.2605, 0.3602) was found between MRI T2* liver and direct bilirubin, indirect bilirubin and alkaline phosphatase (P=0.0024, 0.0089, 0.0002). A weak positive correlation (r=0.2578) was observed between MRI T2* Liver and MRI T2* Heart (Table-2).

DISCUSSION

Transfusion induced iron overload is one of the major complications in patients with β-thalassemia major leading to organ damage and eventually death. Iron accumulation in heart leads to cardiac dysfunction, cardiomyopathy and heart failure, which is the most common cause of death in these patients.[13] The liver is also susceptible to early iron deposition. Liver iron overload, if not diagnosed and properly treated, rapidly develops into cirrhosis. Hence an accurate measurement of liver and cardiac iron level is necessary for the effective use of chelation drugs. Parameters used to monitor iron load include serum ferritin, liver biopsy and MRI assessment of liver and cardiac iron. Liver biopsy is an invasive procedure associated with risk of complications and is subject to sampling error. Hence, serum ferritin and MRI offer valuable means of assessing iron overload due to their ease of measurement and wide availability. Serum ferritin has been the method of choice within the clinic as it is easy to assess, it is inexpensive, and it provides repeat serial measures that are useful for monitoring chelation therapy. It has positive, although imprecise correlation with transfusion burden, morbidity and mortality allowing longitudinal follow-up of patients. It correlates with cardiac impairment and survival[14] but can be elevated by many confounding factors such as infections, inflammation or malignancy. Nevertheless, the close relationship between serum ferritin and survival and its relative ease of measurement makes it the most practical parameter for sequential monitoring.

In contrast MRI technology has enabled accurate and specific measurement of organ iron load, providing a more efficacious means of tailoring therapy to individual risk, but is expensive and not easy for examination in children. Studies have revealed controversial results on the association of serum ferritin levels with MRI findings. In a study on 106 patients with β-thalassemia, a negative association was reported by Voskaridou *et al*, [6] between serum ferritin levels and cardiac iron storage level in MRI. Zamani *et al*, [8] found a moderate negative correlation between serum ferritin and liver MRI T2 *. Tony *et al*,[9] did not find a strong association between serum ferritin levels and cardiac MRI T2. In the research of Majd *et al*, [15] a significant negative correlation was observed between serum ferritin levels and heart and liver MRI T2*. Azarkeivan *et al*,[16] found no significant correlation between serum ferritin level and cardiac MRI T2*, however a significant correlation was observed between serum ferritin and liver MRI T2. A study by Taghizadeh *et al*,[17] on 52 patients with β-thalassemia major revealed a significant negative correlation between serum ferritin levels and liver MRI whereas no correlation was found between serum ferritin levels and cardiac iron levels.

In the present study, a statistically significant moderate negative correlation was found between average serum ferritin and liver MRI T2*. Also, there was a weak negative correlation between average ferritin levels and T2* MRI Heart which was statistically significant. A weak positive correlation was observed between MRI T2* Liver and MRI T2* Heart suggesting that as the iron overload in liver increases iron overload in heart also increases. Our study is consistent with the findings made by Voskaridou *et al*, Zamani *et al*, Majd *et al*,[6,8,15]suggesting that serum ferritin levels can be considered as an indicator to assess iron overload in liver and heart in thalassemia major patients where MRI T2* is not available. Further, as there was significant negative correlation between MRI T2* liver and SGPT and SGOT and positive correlation between MRI T2* and direct bilirubin, indirect bilirubin and alkaline phosphatase these parameters can also be used as to assess iron overload in liver.

CONCLUSION

Accurate measurement of heart and liver parenchymal iron load would enable better treatment outcomes in β-thalassemia patients with iron overload. A significant correlation between serum ferritin and MRI observed in the present study would enable us to use serum ferritin as a parameter to monitor iron overload in patients with β-thalassemia major so that chelation can be initiated at an early stage to avoid the complications of iron overload.

Limitations of this study: As chelation therapy is given when needed, the results are confounded because of therapy (which cannot be withheld for ethical reasons) and the correlations found between serum ferritin levels and MRI T2*liver and heart are perhaps subdued.

Acknowledgements

We are grateful to all the thalassemia patients who participated in the study by giving their written consent. Our special thanks to Chandrakant Agarwal, President, TSCS and all our board members for providing financial support to conduct this study. We are also thankful to our staff without whose effort this project wouldn't have been possible.

Competing interests: The authors have no conflict of interest.

Key Points

- Significant negative correlation was found between average serum ferritin and MRI T2* values of liver and heart
- MRI T2* suggests that the iron overload in liver increases iron overload in heart consecutively
- Serum ferritin levels can be considered as an indicator to assess iron over load in thalassemia patients

Abbreviations

- β-thalassemia Major (β-TM)
- Complete blood count (CBC)
- Ejection Fraction (EF)
- Fetal Hemoglobin (HbF)
- High Performance Liquid Chromatography (HPLC)
- Magnetic Resonance Imaging (MRI)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)

REFERENCES

1. Angastiniotis, M. & Modell, B. Global epidemiology of hemoglobin disorders. Ann. NY Acad. Sci. 1998; 850:251–269.
2. Vichinsky EP. Changing patterns of thalassemia worldwide. Ann N Y AcadSci; 2005; 1054:18-24.
3. Riaz H, Riaz T, Khan MU, *et al*. Serum ferritin levels, socio-demographic factors and desferrioxamine therapy in multi-transfused thalassemia major patients at a government tertiary care hospital of Karachi, Pakistan. BMC research notes; 2011; 4:287
4. Porter, J.B. & Davis, B.A. Monitoring chelation therapy to achieve optimal outcome in the treatment of thalassemia. Best Practice and Research in Clinical Haematology,2002: 15:329–368.
5. Anderson, L.J., Holden, S., Davis, B.*et al*.Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. European Heart Journal,2001; 22:2171–2179.
6. Voskaridou E, Douskou M, Terpos E, *et al*. Magnetic resonance imaging in the evaluation of iron overload in patients with beta thalassemia and sickle cell disease. British Journal of Haematology2004; 126(5):736–42.
7. Stanton K, Geme S, Behrman. Nelson Textbook of Pediatrics: Gastrointestinal Diseases. 19th ed. Iran: ArtinTeb Publishers:2011; 234.
8. Zamani F, Razmjou S, Akhlaghpoor S *et al*. T2* magnetic resonance imaging of the liver in thalassemic patients in Iran. World J Gastroenterol; 2011; 17(4): 522–25.
9. Tony S, Daar S, ElshinawyM, *et al** MRI in regularly transfused children with thalassemia intermedia: serum ferritin does not reflect liver iron stores. PediatrHematolOncol; 2012; 29(6): 579-84.
10. Argyropoulou MI, Astrakas L. MRI evaluation of tissue iron burden in patients with β-thalassaemia major. PediatrRadiol; 2007; 37(12):1191–1200.
11. Au W, Lam W, Chu W, *et al*. A T2* magnetic resonance imaging study of pancreatic iron overload in thalassemia major. Haematologica/ Hemato J; 2008; 93(1):116-19
12. Leung AW, Chu WC, Lam WW, *et al*. Magnetic resonance imaging assessment of cardiac and liver iron load in transfusion dependent patients. Pediatr Blood Cancer. 2009; 53(6):1054–9
13. Baksi AJ, Pennell DJ Randomized controlled trials of iron chelators for the treatment of cardiac siderosis in thalassemia major. Front Pharmacol; 2014; 5:217
14. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood.;1997; 89: 739-61.
15. Majd Z, Haghpanah S, Ajami GH, *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(4): 249-59.
16. Azarkeivan A, Hashemieh M, Shirkavand A, *et al* Correlation between Heart, Liver and Pancreas Hemosiderosis Measured by MRI T2* among Thalassemia Major Patients from Iran. Archives of Iranian Medicine; 2016; 19(2): 96-100.
17. Taghizadeh Sarvestani R, Moradveisi B, Kompany F, *et al*. Correlation between heart and liver iron levels measured by MRI T2* and serum ferritin in patients with β-thalassemia major. Int J Pediatr; 2016; 4:1559-67

****