Correlation between Heart and Liver Iron Levels Measured by MRI T2* and Serum Ferritin in Patients with β-thalassemia Major

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Abstract

Background
Transfusion-induced iron overload leads to many complications in patients with β-thalassemia major. This study aimed to compare the level of iron stored in the liver and heart measured by MRI T2 * with ferritin levels in these patients.

Materials and Methods
This study was done on 52 patients with β-thalassemia major aged 7 to 29 years. Serum ferritin level was checked and heart and liver MRI T2* operation was performed, then the liver and heart iron level measured by MRI T2 * was compared with serum ferritin levels.

Results
150-180 cc/kg packed cells were received on average. The mean ± SD serum ferritin level was 2644.8±1988.3 ng/mL. The mean ± SD relaxation times in liver and heart MRI T2* were 4.39±5.8 and 26.59±10.62 millisecond, respectively. A negative significant correlation was found between serum ferritin levels and liver MRI T2* ($P<0.05$, r= -0.374). However, no statistically significant association was observed between serum ferritin levels and heart MRI T2*($P>0.05$).

Conclusion
Since there was no correlation between serum ferritin levels and cardiac iron levels, accurate assessment of cardiac iron load using MRI T2* is necessary in patients with β-thalassemia major. Serum ferritin level is reliable for assessing liver iron levels.

Key Words: β-thalassemia, Ferritin, MRI T2*, Iron overload.

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1- INTRODUCTION

Thalassemia is a hemolytic hypochromic microcytic anemia characterized by the congenital disorder in hemoglobin production. It is estimated that 3% of the world's population are carrier of β-thalassemia trait. This disease is the most common genetic diseases in the world (1, 2). It is predicted that in the next 20 years, about 900,000 patients with thalassemia will be born in the world, 95% of them will be in Asia especially in India and Middle East (3). Thalassemia involves mainly the under-developed countries and the prevalence of the disease is less in the Europe and North America (4, 5).

According to the Iranian Thalassemia Society, the number of patients with thalassemia was 18,616 in 2005, this number is more than 20,000 in 2016 and most of these patients are in Mazandaran and Fars provinces-Iran (6, 7). This disease is inherited from parents in the form of autosomal recessive and the probability of infant with β-thalassemia is almost one case in 300 deliveries in Iran. This is 3-times more than the highest frequency of inherited diseases like down-syndrome with prevalence of 1 in 1000 cases (8).

Patients with β-thalassemia major need life-long transfusions, without which they would suffer from severe anemia (hemoglobin<6 gr/dlit) (9). A common clinical problem in these patients treated with blood transfusion is iron overload in different organs, such as heart, liver, endocrine glands, pancreas, lungs, and kidneys (1, 2). Each unit of blood contains 200-250 mg of iron (10, 11). Iron overload is toxic to the organs, especially for heart and endocrine glands (2).

The liver is the first organ to store iron overload, saving almost 70% of the body's visceral iron. Therefore, liver iron reflects total body iron. Heart is another important organ that store's iron. Investigating cardiac iron overload is necessary since it is the major cause of death in patients with thalassemia (9). Heart anatomy changes due to iron overload include atriums and ventricles dilation and myocardial thickness increase of 2 to 3 times. Heart failure and cardiac arrhythmia are also the mortal complications in the patients with β-thalassemia major (12).

Serum ferritin is used as a laboratory test for measuring the body's iron storage. However, ferritin is not indicative of the iron storage level of the whole body, especially in cases such as inflammation and liver diseases in which serum ferritin levels rise without increasing the iron load (10). Moreover, iron load stored in the liver is not significantly associated with cardiac iron overload. Moreover, in cases that the decisions are based solely on the patient's serum ferritin level, too little or too much chelation therapy could be received. Therefore, a safe method for estimating total body iron is required to evaluate the adequacy of chelation therapy (2). Among the existing methods for determining the body's iron level in thalassemia patients, T2* Magnetic Resonance Imaging (MRI) is a selective, non-invasive, and safe method (11, 13).

MRI is a test that uses a magnetic field and pulses of radio wave energy to make pictures of organs and structure inside the body. T2* relaxation refers to decay of transverse magnetization caused by a combination of spin-spin relaxation and magnetic field inhomogeneity. Transverse magnetization is formed by tilting the longitudinal magnetization into the transverse plane by using a radiofrequency pulse. A characteristic time representing the decay of the signal by $1/e$, or 37%, is called the T2* relaxation time. The detailed description of the MRI T2* is beyond the scope of this study. References (14-16) provide detailed information on this matter.

Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels were among the tests used for measuring the liver function. Levels of both AST and ALT signals were elevated in early liver injury in patients with high liver iron storage (17).

Recently, studies have been done on different aspects of using MRI to determine the patients' iron overload and its association with serum ferritin level (17-20); however, the results are still controversial. In a study on 106 patients with thalassemia, a negative association was found between serum ferritin levels and the reported cardiac iron storage level in MRI (18). Other researchers did not find a strong association between serum ferritin levels and cardiac MRI T2*(19). Zamani and colleagues found a moderate negative correlation between serum ferritin and liver MRI T2 * (20).

In the research of Majd and colleagues, a significant negative correlation between serum ferritin levels and heart and liver MRI T2* was observed (21). Azarkeivan and colleagues 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*(22). Considering the differing results reported in different studies, we aimed to compare the level of iron stored in the liver and heart measured by MRI T2* with ferritin levels in patients with β-thalassemia major. Accurate measurement of heart and liver parenchymal iron load is required since the estimation of heart and liver iron load in patients with thalassemia major is necessary for better treatment outcomes.

As mentioned above, it is important to evaluate the treatment adequacy of patients by performing MRI T2*. In this study, the treatment adequacy of patients with thalassemia was evaluated using MRI and the correlation between serum ferritin and the reported body iron level in liver and heart MRI T2* was determined to establish whether ferritin could be a suitable index for assessing treatment adequacy in such patients. In children, MRI is not an easy examination; therefore, one of the goals of this study was to found an easier method. If a significant correlation could be found between MRI finding and ferritin level, ferritin can be used to follow the patients easily and inexpensively.

2- MATERIALS AND METHODS

The study was carried out on 52 patients with thalassemia major, aged 7-29 years referred to Besat Hospital in Sanandaj, Kurdistan-Iran, during 2012-2013 (*Ethical code*: REC.MUK.1392.98).

All patients treated with regular blood transfusions (more than 8 times per year) from three years prior to the study), were enrolled, after approval of the study which did not impose any financial burden on the patients. These patients received blood transfusions every three to four weeks with an average amount of received packed cell of 180-150 cc/kg. The patients' serum ferritin, AST, and ALT levels were checked. Ferritin was measured using the ELISA device (Anthos 2020 model, Austria) based on ng/mL and ALT and AST was measured by the automatic analyzer device (HITACHI 902, Japan) based on mg/dL. MRI T2* was used to determine the patients' cardiac and liver iron load using the MRI device (ESSENZA 1.5 Tesla model, Germany). In MRI T2*, relaxation time shows the severity of iron overload in the heart and liver, with differing numbers (23) (Tables 1, 2).

Then the ferritin level was compared with the MRI T2* reported iron storage level (as gold standard for showing the body's iron storage level) to evaluate the association between serum ferritin with the reported liver and heart iron in MRI T2*. On the other hand, AST and ALT serum

levels were measured and the effect of liver iron overload level on AST and ALT levels was evaluated. Confounding factors such as hepatitis and fatty liver that affect AST and ALT results were checked by liver ultrasound (MEDISON ultrasound device, ACUVIX V10 model made in Korea) and A, B, and C hepatitis tests were done using ELISA READER device (Bio Tek ELX800, USA) and leading medical kits (Lot No: 92006, 92007, 92004, Iran).

In the cases where patients were not satisfied that they had participated in the study, irregular blood transfusions (uncertain amount and irregular intervals), reports of sonography with increased hepatic echogenicity and positive hepatitis A, B or C, the patients were excluded from the study; also patients with fatty liver or hepatitis were excluded. It is worth noting that age was not critical for this study. Then, AST/ALT serum levels of the remaining patients were compared with the reported liver iron level in MRI T2* to evaluate the correlation of liver iron overload with the increase or decrease of liver enzymes. Data were analyzed using SPSS software, version-11.5, using Spearman's correlation coefficient. After univariate analysis, multivariate analysis was using multiple regressions and factors effecting liver iron load level were determined.

Table 1: Relaxation times for showing the severity of heart iron overload

Myocardial loading	Myocardial T_2^* r(ms)
Normal	≥20
Mild	14-19.99
Moderate	10-13.99
Severe	≤ 9.99

ms: millisecond

Table 2: Relaxation times showing the severity of liver iron overload

Hepatic loading	Hepatic T_2^* (ms)
Normal	≥6.3
Mild	2.8-6.29
Moderate	1.4-2.79
Severe	≤1.39

3- RESULTS

52 patients, 23 women (44.2%) and 29 men (55.8 %) were involved in the study. The patients' mean ± SD age was 17.3±7.3 years and their mean ± SD serum ferritin level was 2644.8±1988.3 ng/mL. The mean ± SD relaxation times in these patients' in heart and liver MRI T2* were 26.59±10.62 and 4.39±5.8 msec, respectively. The relaxation time of cardiac MRI was respectively ≥ 20 msec in 37 (71.2 %) patients, 14-19.9 msec in 8 (15.4%) patients and 14> msec in 7(13.5%) patients. There was no significant correlation between serum ferritin and the reported iron level in heart MRI T2* (P=0.161, r = -0.197), (Figure.1).

Among the studied patients, the reported liver iron level was respectively normal in 8 patients (15.4%), mild iron overload in 15 patients (28.8%) and moderate to severe iron load in 29 patients (55.8%). There was a significant negative correlation between serum ferritin level and the reported iron level in liver MRI T_2^* (r = -0.374, P=0.006), (Figure 2 and Table.3). Sonography with increased hepatic echogenicity was reported in 4 (9.6%) patients, positive hepatitis B or positive hepatitis C in 5 (11.5%) patients and both increasing echogenicity and hepatitis in one patient. Therefore, 10 patients were excluded from the study when the correlation among AST and ALT with MRI and with ferritin were considered. A statistically significant correlation was found between AST and ferritin levels (r= 0.506, P=0.001) and also ALT and ferritin levels (r= -0.378, P=0.013), (Figure.3 and Table.3). A statistically significant correlation was seen between the reported iron level of liver MRI T2* and AST level (r= -0.370, P=0.016) and ALT (r= -0.502, P=0.001), (Figure.4). There was a statistically significant correlation between cardiac MRI and liver MRI (r=0.292, P=0.036), (Table.4). This table shows the number of

patients categorized in the groups of cardiac MRI and Liver MRI. Also, a statistically significant correlation was seen between cardiac MRI T2* and age (r=0.356, P=0.01), (Table.5), while no such correlation was observed between liver MRI T2* and ages. Age (r=0.412, P=0.054) and ferritin level (r=-0.002, P=0.032) were associated with cardiac iron load level in the multivariate analysis and the constant coefficient was 22.985 (R2=0.193).
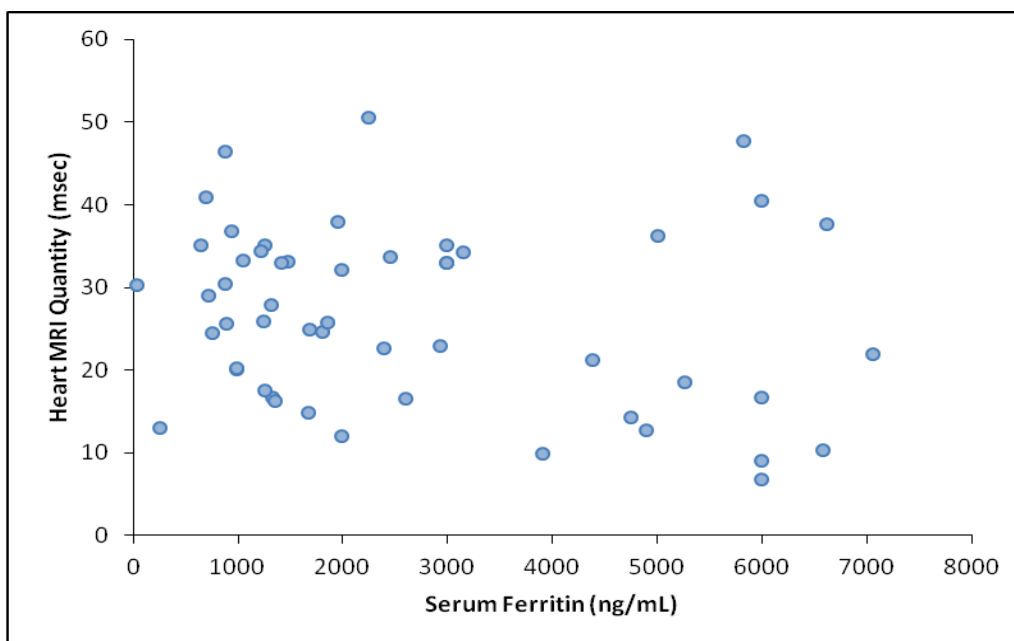

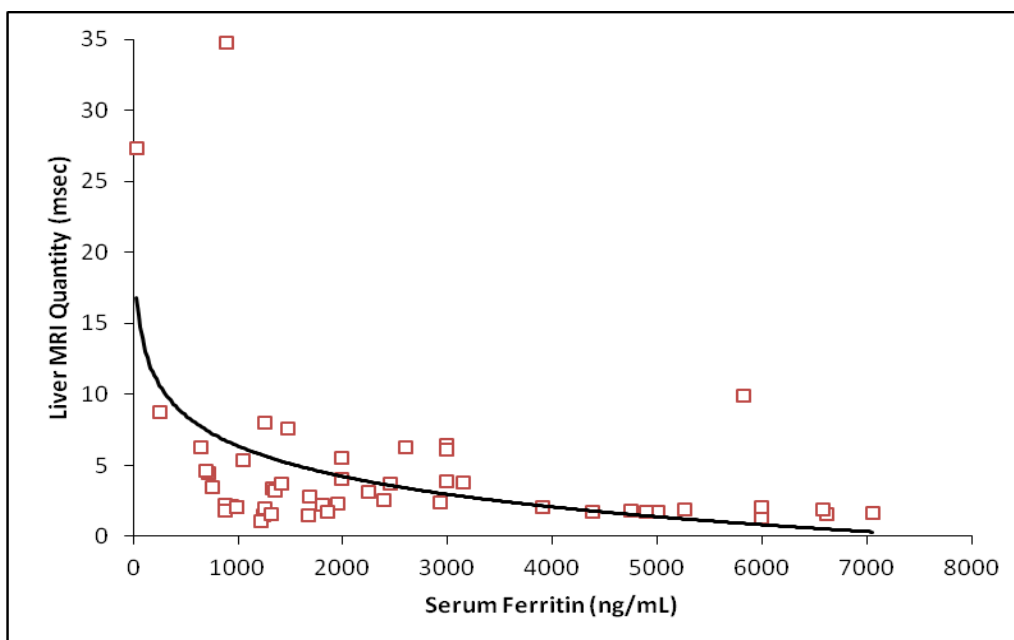**Fig.1:** Correlation between serum ferritin level and cardiac MRI

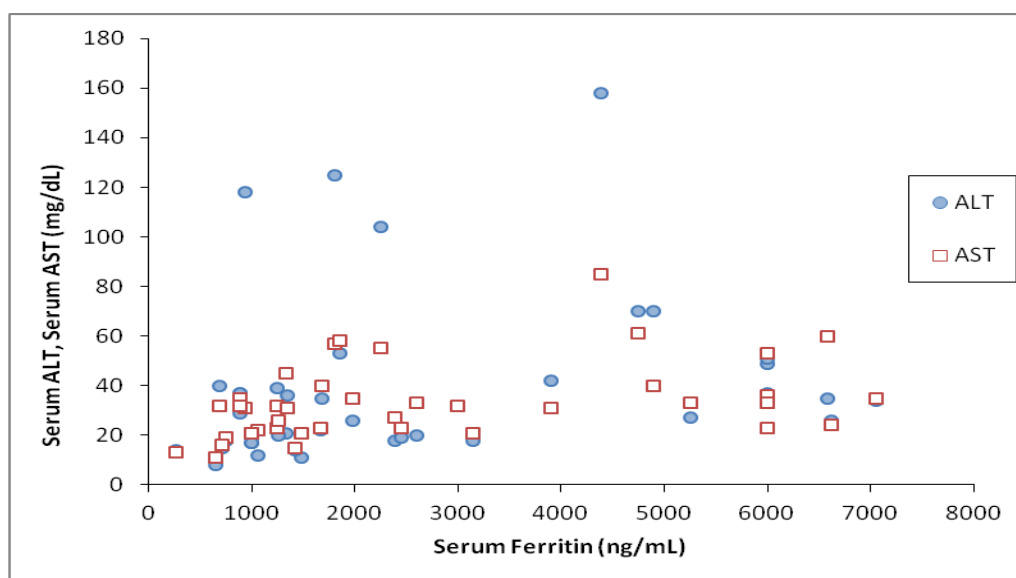**Fig.2:** Correlation between ferritin serum level and liver MRI

Fig. 3: Correlation between ferritin serum level and ALT and AST serum
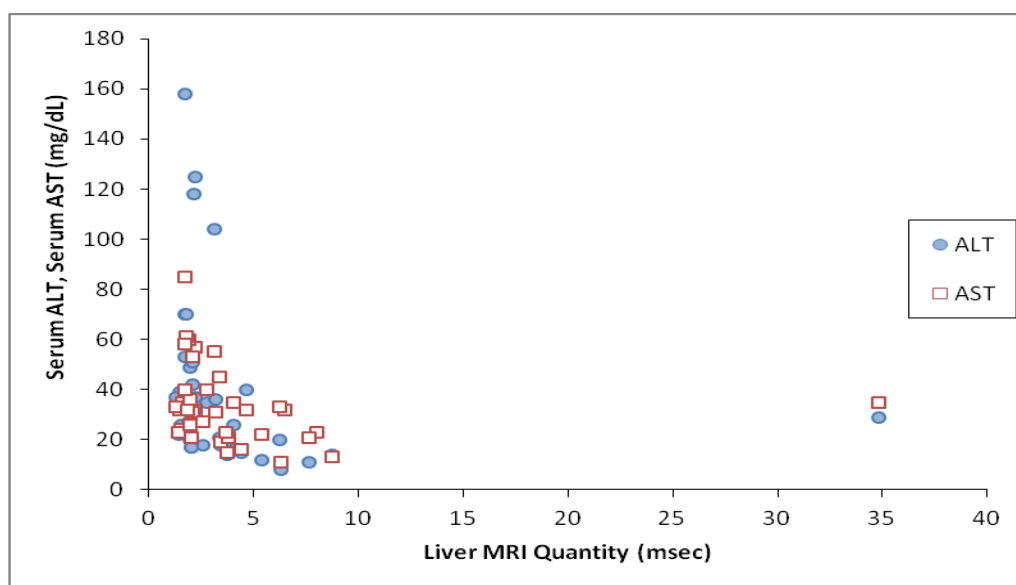

Fig.4: Correlation between liver MRI and ALT and AST serum level

Table 3: Correlation between serum ferritin level and other indices

Variable	AST Correlation coefficient	ALT Correlation coefficient	Cardiac MRI Correlation coefficient	Liver MRI Correlation coefficient	Liver MRI** Correlation coefficient
Ferritin (ng/ml)	0.506*	0.378*	-0.197	-0.374	-0.522*
P- value0.001		0.013	0.16	0.006	0.001

* 10 patients were excluded because of hepatitis or increased liver echogenicity;
** Excluding the patients with hepatitis or increased hepatic echogenicity, a more significant statistical negative correlation was observed between ferritin level and liver MRI (r= -0.522, P<0.05)

Table4: Number of patient categorized in groups of cardiac MRI and liver MRI

Liver MRI (ms) Cardiac MRI (ms)	≥6.3 (Normal)	2.8-6.29 (Mild)	1.4-2.79 (Moderate)	≤1.39 (Severe)	Total
≥20 (Normal)	7	11	18	1	37
14-19.99 (Mild)	0	3	5	0	8
10-13.99 (Moderate)	1	1	2	0	4
≤9.99 (Severe)	0	0	2	1	3
Total	8	15	27	2	52

Table5: The statistical correlation between age and the other variables

Variable	Ferritin (ng/ml)	Cardiac MRI (ms)	Liver MRI (ms)
Age (year)	0.071	0.356	0.139
P- value	0.618	0.010	0.326

4- DISCUSSION

At current study, there was no statistically significant correlation between ferritin level and the reported iron level in cardiac MRI T2*, consistent with another similar study (10).

On the contrary, a significant negative correlation was reported between ferritin level and cardiac iron storage level in MRI of patients with thalassemia major in a study done by Voskaridou and his colleagues (18) and these different results showed the necessity of performing more studies on such patients. Since cardiac iron overload is highly associated with mortality in patients with thalassemia, an accurate measurement of cardiac iron level is necessary to use chelation drugs. Based on our findings, it is necessary to perform cardiac MRI T2* annually since serum ferritin level cannot is not necessarily indicative of cardiac iron level. Since liver is the first organ to store iron in the body, most of the patients had moderate liver iron overload in the study. A significant negative correlation seen between ferritin level and the reported iron

level of liver MRI T2*. Therefore, it could be inferred that a higher level of serum ferritin is indicative of a higher liver iron level and vice versa. Other studies have shown a significant correlation between ferritin level and the reported iron level of liver MRI T2* (18-20). Moreover, we found a significant positive correlation between AST and ALT levels and the serum ferritin level. A statistically significant correlation was seen, between cardiac MRI and liver MRI. Therefore, it could be concluded that increase in liver iron storage leads to higher levels of cardiac iron. Most participants had moderate MRI liver iron level and normal cardiac MRI. Since liver is the first organ to store iron in the body, most patients' had moderate iron levels on liver MRI. Age was significantly correlated with cardiac MRI T2*, but not with liver MRI T2*. Cardiac involvement usually increases in patients over 10 years of age with increase in blood transfusions, so cardiac iron overload increases as people age. The clinical application of these findings is that considering the correlation

between serum ALT, AST, and ferritin levels with liver MRI T2*, they can be used as an index for liver iron overload. Also, ferritin could not be used as a measurement of cardiac iron overload because there was no correlation between ferritin and cardiac MRI T2* and since the assessment of cardiac iron load level and the outcome of thalassemia major is necessary to treat patients, cardiac parenchyma iron load should be measured accurately using MRI T2*.

5- CONCLUSION

No correlation was seen between ferritin and cardiac iron storage and hence it is necessary to measure cardiac iron load by MRI T2* in patients with β-thalassemia major accurately. However, ferritin serum level is reliable to evaluate the liver iron level. Since these tests are more accessible and less expensive, they can be used for following patients to prevent further liver damage caused by iron overload.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENTS

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