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Renal Iron Load Estimation in Thalassemia Patients Using T2* Magnetic Resonance Imaging

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ABSTRACT

Purpose: *Multi-organ hemosiderosis is a known complication in thalassemia patients with chronic blood transfusion. T2-Star (T2*) MRI has been introduced as a non-invasive tool for detecting iron overload in the liver and heart in these patients. This study is to determine and assess renal iron overload by MRI and its relation to liver and heart iron and serum ferritin in Iranian thalassemia patients.* ***Methods:*** *Total 821 transfusion dependent major and intermediate thalassemia patients (age range 10-50 years) were included in this study and calculations were done on their MRI data in a medical imaging center through 2014-2016. Iron values were calculated and averaged in a different region of interests (ROI) using fast-gradient-echo multi-echo T2* sequences.* ***Results:*** *Pathological renal iron content less than 36 ms was around 19.6%. The mean T2* kidney of the total population was 50.26 ms. A moderate negative, statistically significant correlation between kidney T2* relaxation time and serum ferritin was noted. For liver and heart, T2* relaxation time weakly, a statistically significant correlation was acquired by renal T2* relaxation time.* ***Conclusions:*** *Renal hemosiderosis was shown in numerous thalassemia patients. Since the frequency of renal iron deposition was approximately 20% in TM patients in a general population study, it might shed light that frequently monitors the renal iron loading merit hematologists in preventing the secondary side effects.*

Keywords: Iron load, Beta-thalassemia, Renal, Magnetic resonance imaging T2*

INTRODUCTION

Thalassemia syndromes are the most prevalent inherited hemoglobin disorders (Hb) worldwide [1-4]. Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by quantitative and to a lesser extent qualitative abnormalities in beta-globin chain synthesis, producing chain imbalance resulting in decreased red blood cells (RBC) and eventually anemia [4]. The phenotypes of homozygous or genetic heterozygous compound beta-thalassemia include thalassemia major (TM) and thalassemia intermedia (TI). Patients with thalassemia major are usually present with profound anemia in early childhood and require regular blood transfusion and iron chelation therapy to survive while thalassemia intermedia patients, present later or remain transfusion independent [4,5].

Because of frequent blood transfusion, iron deposition occurs in visceral organs such as the heart, liver, endocrine glands, kidney, etc., causing tissue damage and ultimately, organ dysfunction or failure. Although chelating therapy considerably improves the survival in patients with chronic transfusion, multi-organ failure secondary to hemosiderosis is still common [4,6,7].

Despite the fact that serial measurements of serum ferritin are an easy and available method for evaluating the iron overload, previous studies have shown that there is a stronger correlation between liver iron concentration and total body iron [8,9]. Nonetheless, liver biopsy is considered as an invasive method and "target" organs like heart or kidney are not tractable to biopsy [4,8,10].

MRI technique (gradient echo $T2^*$ and T2 spin echo) for assessing iron burden in the liver and heart was developed in the early 1980s. Hemosiderin molecules produce local disturbances in the magnetic field; greater organ iron content causes increased magnetic field disturbance. This means that the presence of iron storage in tissues leads to reduced field homogeneity and a low $T2^*$ signal is recorded [3,8-12]. The introduction of MRI (gradient echo $T2^*$ and T2 spin echo or R2 relaxometry) to quantify liver and cardiac iron had a profound impact on our understanding and management of iron overload in thalassemia patients, treatment modifications and prevention of cardiomyopathy caused by iron overload [8,13-17]. Worldwide multi-center studies have shown that the result of myocardial $T2^*$ is a powerful prognostic factor for cardiac complications, it has the potency for further development in favor of designing optimal chelation regimens for prevention of heart failure, and prolong survival [13,18].

Roubicloux in 1994 showed that there was low signal intensity in the renal cortex on T2-weighted images in severe hemolytic anemia due to iron deposition [19].

Since pathophysiology, mechanism, and rate of iron overload, seems to be different in thalassemic patients' affected organs (transfusion dependent and non-transfusion dependents), diagnosis of iron overload, quantifying and monitoring of their exclusive extent might really benefit patients [20].

However, there is very little information on renal involvement in thalassemia patients. According to a few studies which have evaluated the kidney involvement in patients with beta-thalassemia, the renal function declines over time [3,21-26]. Based on these studies, renal dysfunction is common in thalassemia patients. Severe iron overload as a consequence of frequent transfusions, overdosages of chelating agents, and to anemia are among the proposed etiologies for the renal dysfunction [24-29].

Previously, few studies were conducted by Hashemieh, et al., and Meloni, et al., and Schein, et al., for assessing hemosiderosis in kidneys in thalassemia patients (TM and TI), in which MRI $T2^*$ techniques were used [3,30,31]. The authors considered the serum ferritin level and the iron overload of the heart and kidney. The study conducted by Hashemieh, et al., study was prospective research, which included 120 TI and TM patients while Meloni, et al., survey enrolled 119 TM patients [3,30]. In a study of Schien and colleagues, MRI was performed in 75 sickle cell patients, 73 TM patients, and 16 healthy controls. Multiecho gradient echo protocols were used to measure $R2^*$ in the kidney, liver, and heart. Kidney $R2^*$ was compared to tissue iron estimates and serum iron markers [10]. In the recent research, the specialists investigated renal function in Iranian transfusion-dependent thalassemia major patients and for achieving this goal did laboratory tests for early markers of glomerular and tubular dysfunction in for these patients [31]. Juanqueria survey was to assess renal iron deposits in patients with sickle cell anemia, correlating these values with transfusion burden, liver and heart $T2^*$ [32].

The aim of this retrospective cross-sectional study is to assess the renal $T2^*$ MRI iron load in Iranian thalassemia as well as to investigate the renal hemosiderosis relation to the serum ferritin, myocardial and hepatic iron overload among sample study of Iranian thalassemia patient.

MATERIALS AND METHODS

Patient Population

This survey was conducted in a referral medical imaging center for thalassemia patients for their annual $T2^*$ MRI (Hemosiderosis assessment) imaging follow-up in Tehran, Iran. Informed consent has been previously obtained for this HIPAA-compliant study. This retrospective study follows the principles of the Declaration of Helsinki and was approved in the ethical committee of the Iranian blood transfusion organization (IR.TMI.REC.1396.023). This study was conducted for the available data from July 2014 till December 2016. A total of 821 Iranian patients of TM and TI who met the inclusion criteria were included in this study. All the patients were blood-transfusion dependent with transfusion duration of 14-30 days depending on the specialist prescription. Patients with age less than 10 years

and more than 50 years were excluded from the study. In addition, we didn't include patients on oral iron chelation therapy regimen in our survey and pregnant patients. The patients almost totally were on iron chelation therapy with Desferrioxamine (Desferal). Demographic data such as age, gender, type of thalassemia, height, weight, recent serum ferritin level, age of diagnosis and splenectomy status were obtained from the medical records.

Magnetic Resonance Imaging

Patients were scanned with a 1.5T MR Scanner (Achieva A-series Philips, Netherland). A standard radiofrequency (RF) body coil was used in all measurements. The Royal Brompton protocol based on a single-breath multi-echo fast gradient-echo sequence was used for $T2^*$ measurements. The calibration procedure in this setup was frequently performed using a standard iron phantom.

The liver and kidney $T2^*$ were determined by imaging a single trans-axial slice (10 mm) through the center of the liver and kidneys. For the measurement of myocardial $T2^*$, scans were synchronized to the cardiac cycle utilizing standard ECG gating. A single 10 mm-thick, short-axis, mid-ventricular slice positioned halfway between the base and the apex of the left ventricle (LV) was acquired. $T2^*$ values were calculated for patients using in-house software (Pardis Noor Medical Imaging Centre, Tehran, Iran). A homogeneous region of interest (ROI) derive was outlined in the liver and kidney parenchyma. A homogeneous full-thickness ROI was chosen in the ventricular septum. The mean signal intensity of the region was measured for each image (Figure 1) and plotted against the echo time (TE). $T2^*$ values were calculated in 3 different ROIs and were averaged to achieve a representative value for the kidney. As the standard threshold of kidney $T2^*$ relaxation time we referred to the value in reference indicating the lower limit of normal kidney $t2^*$ based on the healthy population data to be less than 36.8 ms [30].

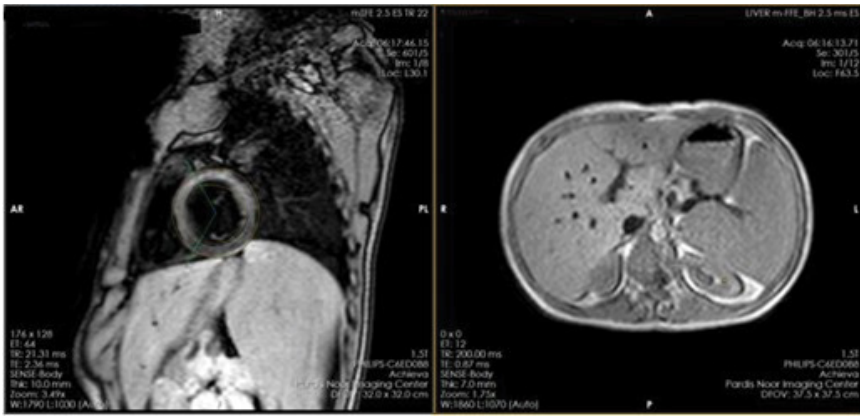

Figure1 MRI $T2^*$ images of cardiac and abdomen cross-section for iron overload processing and calculation

Statistics Analysis

Descriptive analyses, including frequencies, means, and standard deviations were calculated in the study population. The assumption of normality was checked. The relationship between kidney as a response and gender and type of thalassemia was found using independent T-test. Pearson's correlation coefficient was used to detect the correlation between the considering variables in the study. Also, the linear relationship of age and serum ferritin as covariates and kidney $T2^*$ relaxation was performed by linear regression analysis.

The level of significance was equal to 0.05 and confidence interval (CI) considered to 95% boundaries. All statistical data analysis was performed using SPSS version 15 software.

RESULTS

Demographic characteristics of patients are described in Table 1. The mean (SD) age of 821 participants was 27.9 (8.6%) years (range 10-50 years) and the mean (SD) ferritin level was 1837 (SD) 1690 ng/mL. The vast majority of the included patients suffered from thalassemia major and others were intermediate thalassemia (634 Vs 187 respectively).

Table 1 Demographic characteristics of blood transfusion-dependent thalassemia patients (n=821)

Variables	Total	Type of Thalassemia		p-value
		Major (n=634)	Intermedia (n=187)	
Sex				
Male N (%)	384 (46.8%)	298 (77.6%)	86 (22.4%)	0.86
Female N (%)	437 (53.2%)	336 (76.8%)	101 (23.2%)	
Splenectomy				
Yes N (%)	427 (51.9%)	303 (70.9%)	124 (29.1%)	<0.0001*
No N (%)	394 (48.1%)	331 (84%)	63 (16%)	
Age (Year)				
Mean	27.9	27.1	31.1	<0.0001*
SD	± 8.6	± 8.1	± 9.5	
Diagnosis Age (Month)				
Mean	38.3	25.1	86.7	<0.0001*
SD	± 60.3	± 35.3	± 97.5	

In our study, the mean kidney $T2^*$ of the involved patients was 50.26 ms (minimum=8.27 Vs maximum=104.3, standard deviation=16.97). About 19.6% of our patients (26 intermedia thalassemia Vs. 135 thalassemia major) had Kidney $T2^*$<36 ms that may indicate kidney hemosiderosis.

Mean kidney $T2^*$ value did not show a significant difference between men and women (men 49.57 ± 16.7 vs. women 50.9 ± 17.2, p=0.26) (Figure 2). However, there was a significant difference between the mean kidney $T2^*$ values of major and intermediate thalassemia (intermediate thalassemia 54.4 ± 17.8 vs. thalassemia major 49.1 ± 16.53, p<0.001).

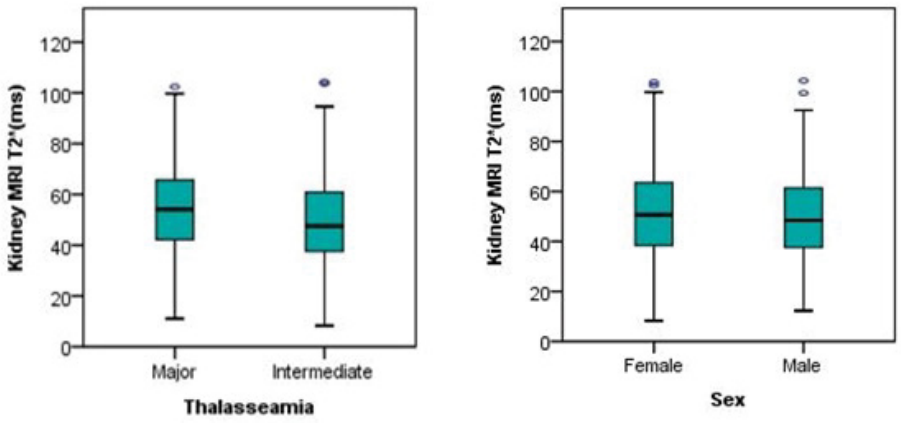

Figure 2 Box plot of kidney $T2^*$ relaxation time and sex and thalassaemic variables separately

A weak inverse correlation was found between serum ferritin levels and kidney $T2^*$ relaxation time values (r=-0.40, p<0.0001). Linear regression analysis found a significant effect of serum ferritin on kidney $T2^*$ relaxation time in spite of weakly correlation. This correlation has also been presented as a scatter plot in Figure 2. The chart showed a reduction pattern in the ferritin values. Liver and heart $T2^*$ relaxation time variables were correlated to kidney $T2^*$ relaxation time with weakly positive correlation (r=0.36, p<0.0001 and 0.31, p<0.0001 respectively). Both liver and heart values followed an increasing pattern in ferritin values (Figure 3). According to results in Table 2 and Figure 3, there was a positive but weak correlation between age and kidney $T2^*$ relaxation time (r=0.14, p=0.005).

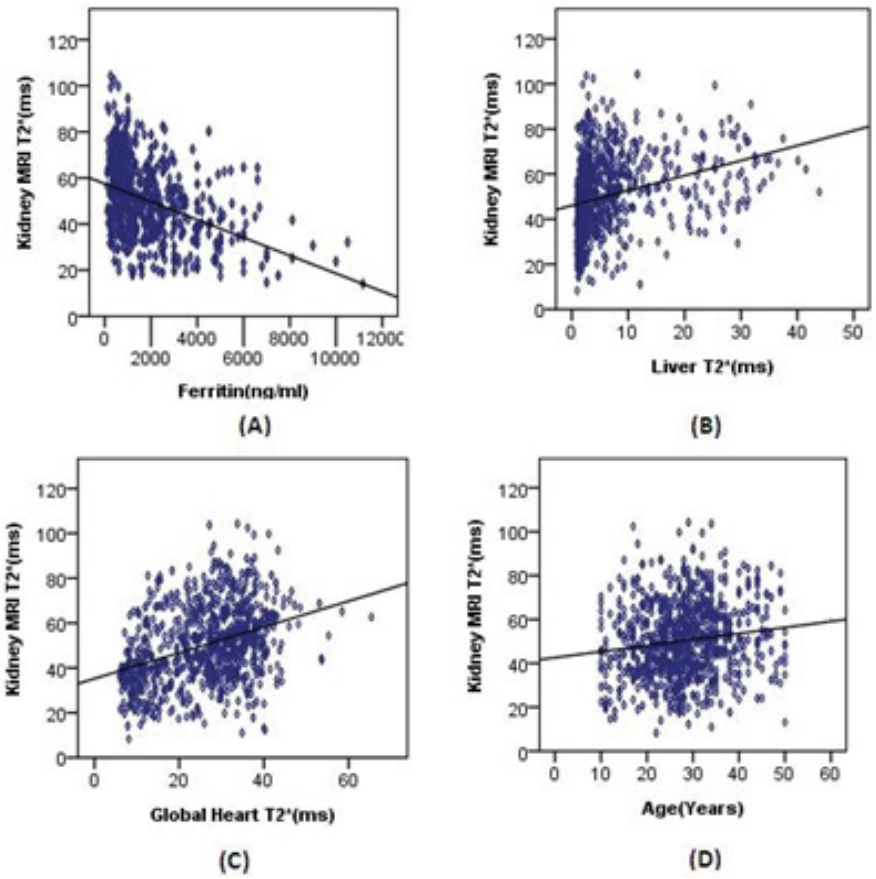

Figure 3 Scatter plot of kidney T2* relaxation time compared with age, serum ferritin, Liver T2*, and Heart T2* variables

Table 2 Linear multivariate regression analysis between kidney T2* relaxation time and independent variables

Variables	Multivariate Regression					
	Pierson (r)	correlation (r^2)	p-value	β	95%CI	p-value
Age	0.14	0.02	0.005*	0.07	-0.284	<0.057
Serum ferritin	-0.4	0.16	<0.0001*	-0.27	-0.001	<0.0001*
Liver T2* relaxation	0.31	0.1	<0.0001*	0.15	0.150-0.490	<0.0001*
Heart T2* relaxation	0.36	0.13	<0.0001*	0.24	0.250-0.480	<0.0001*
β: standardized coefficient Beta; CI: Confidence interval; *Significant level (p<0.05)						

DISCUSSION

Beta thalassemia is the most prevalent genetic disease widely distributed throughout the Mediterranean, Middle Eastern and Asian countries [2]. Although supportive therapies such as regular transfusion and iron chelation have significantly improved prognosis and the life expectancy in beta-thalassemia patients, they cannot stop the disease or the therapy-related complications like iron loading in vital organs such as heart, liver, pancreas, kidney [2,6]. Since the introduction of MRI T2* as a technique to assess iron overload in the tissues, several extensive surveys were reported for heart and liver hemosiderosis patterns but there was a paucity of data for using T2* relaxation time for the kidney [8,13-18]. The goal of this study was to investigate the frequent distribution (%) of iron loading in the kidney in a large population of beta-thalassemia patients (TM, TI). We mainly studied the correlation of renal hemosiderosis with selected factors like age, serum ferritin, and liver and heart iron overload based on their MRI T2* relaxation times.

Our results showed a weak positive linear but not statistically significant relationship between age and kidney relaxation T2* time, which indirectly reflects years of transfusion therapy (Table 2 and Figure 3). This finding is in concordance with the results of studies by Hashemieh, et al., and Koliakos, et al., that reported no statistically significant correlation

between age to kidney $T2^*$ relaxation time [3,6]. Moreover, we found a moderate negative, statistically significant correlation between kidney $T2^*$ relaxation time and serum ferritin as there has been concluded by Hashemieh, et al., for a moderate correlation. Our results also indicated a weak uphill linear relationship, statistically significant correlations between kidney $T2^*$ relaxation time and liver and heart $T2^*$ relaxation time. Although by Hashemieh, et al., such a similar result has been reported, in Schein study, it has been presented that kidney $R2^*$ failed to correlate with iron deposition in liver or heart tissues [10].

Based on our study results, we estimated the frequency distribution (%) of kidney, heart and liver iron overload in the included patients. In the results presented in Figure 3, it was seen that based on the majority, data of the included thalassemia patients, 70.9% have normal range of myocardial iron content while only 30.7% of them have normal hepatic iron hemosiderosis. These results seem to dictate a discrepancy in myocardial and hepatic hemosiderosis pattern. It also proposed that there might be different mechanisms and kinetics of iron uptake, storage, and clearance in various tissues such as heart, liver, kidney, and pancreas [3,8]. Then, it may be a useful suggestion to merit renal MRI $T2^*$ imaging for estimating its iron loading. Total 161 patients (19.6% of population study) in our study had kidney iron overloading values which represent some degree of renal iron hemosiderosis in our study population while kidney iron overload $T2^*$ relaxation time trends were similar in both thalassemia major and intermedia groups. This seems to be in accordance with results of Meloni, et al., study that reported about 33.6% of total enrolled TM patients had a pathological value ($T2^*$<36.8 ms) of kidney iron deposition and also Schein study that the TM patient demonstrated little kidney darkening or tissue which is an indication of hemosiderosis [10,30]. Last not least, renal hemosiderosis might make into account as a frequent effect due to blood transfusion among major patients, who necessitate regular screening with early markers of glomerular and tubular dysfunction, and it seems to be in accordance with the conclusion of the research showed asymptomatic renal dysfunction in among transfusion-dependent β-thalassemia major patients [31].

We acknowledge a number of limitations to our study. As other available references, we also had limitation to include synchronized tests to investigate the exact correlation of renal iron overload severity estimation using MRI $T2^*$ in thalassemia patients since there was research available on this issue. We also exclude patients who consume oral iron chelator for preventing probable biases. Hence, another extent clinical study is suggested for advance evaluations. Moreover, in this study, we only take advantage of MRI $T2^*$ iron overload calculations. However, for other researches, application of R2 relaxometry measurements employing appropriate MRI protocols might be of value in the non-invasive assessment of kidney iron overload in patients with thalassemia to investigate whether it provides more-reliable tissue iron overload estimations compared to $T2^*$ measurements or not. Due to the high-cost low-availability quantitative iron assessment of invasive kidney biopsy, we also had an obstacle for performing and including.

Few studies have been published till now for kidney iron overload assessment in thalassemic patients, in comparison of pool data of myocardial, hepatic MRI $T2^*$ iron assessment, so such clinical research with larger patient numbers as an Iranian population study was conducted.

Since the percentage of renal iron deposition was approximately 20% in TM patients in a general population study, it might shed a light that frequently monitors the renal iron loading merit hematologists as a clue for the secondary side effects might cause renal dysfunction. Since physiopathology of iron overload in kidney, heart, and liver seems to be different, quick, non-invasive, accessible MRI $T2^*$ technique for estimating of iron overload might merit clinicians to annual monitoring of renal iron burden in thalassemic patients in order to optimize iron chelating regimen of patients and preventing its toxicity effects in the kidney.

CONCLUSION

- Iron loading in vital organs such as heart, liver, pancreas, and kidney is a secondary effect in transfusion-dependent thalassemic patients
- MRI is a non-invasive imaging modality to iron overload quantification
- MRI $T2^*$ imaging method now is safely used for thalassemic patients liver and heart hemosiderosis monitoring
- Accessible MRI $T2^*$ technique merit clinicians for estimating of renal iron burden in thalassemic patients

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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