Published in final edited form as:

J Magn Reson Imaging. 2011 June ; 33(6): 1510–1516. doi:10.1002/jmri.22553.

Reduced Transverse Relaxation Rate (RR2) for Improved Sensitivity in Monitoring Myocardial Iron in Thalassemia

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Abstract

Purpose—To evaluate the reduced transverse relaxation rate (RR2), a new relaxation index which has been shown recently to be primarily sensitive to intracellular ferritin iron, as a means of detecting short-term changes in myocardial storage iron produced by iron-chelating therapy in transfusion-dependent thalassemia patients.

Materials and Methods—A single-breathhold multi-echo fast spin-echo sequence was implemented at 3T to estimate RR2 by acquiring signal decays with interecho times of 5, 9 and 13 ms. Transfusion-dependent thalassemia patients ($N = 8$) were examined immediately before suspending iron-chelating therapy for 1 week (Day 0), after a 1-week suspension of chelation (Day 7), and after a 1-week resumption of chelation (Day 14).

Results—The mean percent changes in RR2, R2 and R2* off chelation (between Day 0 and 7) were $11.9 \pm 8.9\%$, $5.4 \pm 7.7\%$ and $-4.4 \pm 25.0\%$; and, after resuming chelation (between Day 7 and

14), $-10.6 \pm 13.9\%$, $-8.9 \pm 8.0\%$ and $-8.5 \pm 24.3\%$, respectively. Significant differences in R2 and RR2 were observed between Day 0 and 7, and between Day 7 and 14, with the greatest proportional changes in RR2. No significant differences in R2* were found.

Conclusion—These initial results demonstrate that significant differences in RR2 are detectable after a single week of changes in iron-chelating therapy, likely as a result of superior sensitivity to soluble ferritin iron, which is in close equilibrium with the chelatable cytosolic iron pool. RR2 measurement may provide a new means of monitoring the short-term effectiveness of iron-chelating agents in patients with myocardial iron overload.

Keywords

MRI; cardiac MR; heart; RR2; thalassemia; iron overload; chelation therapy; ferritin; hemosiderin

Introduction

Thalassemia, the most common human monogenic disease, is caused by impaired and imbalanced production of globin, the protein component of hemoglobin (1–2). In severely affected individuals, treatment of the resultant anemia requires regular red blood cell transfusion beginning in infancy. Because the body is unable to effectively excrete excess iron, the iron within transfused red blood cells progressively accumulates, eventually injuring the heart, liver, pancreas and other organs (3). Overall, two-thirds or more of patients with thalassemia major die of iron-induced cardiomyopathy (4).

In thalassemia patients with transfusional iron overload, iron-chelation to remove the excess iron is the most direct therapeutic approach (5–6). To prevent iron toxicity from inadequate chelation therapy and avoid the adverse effects of excessive chelator administration, a quantitative means of measuring myocardial iron that is sensitive, safe and non-invasive is needed to improve the management of iron-chelating therapy in transfusion-dependent thalassemia (7). The use of magnetic resonance imaging (MRI) methods to detect cardiac iron deposition in the heart has been a crucial advance in the care of patients with transfusional iron overload (3,8–9). In particular, measurement of the myocardial effective transverse relaxation time ($T2^* = 1/R2^*$) is a strong predictor of the risk over one year of cardiac failure and arrhythmia in patients with thalassemia major (10). Nonetheless, changes in myocardial R2* occur at a slow pace, over periods of several months, even with continuous intravenous therapy with the iron chelator, deferoxamine, while cardiac function improves over periods of weeks (11).

The delayed response of myocardial R2* to the effects of iron-chelating therapy results from differences in the effects of the major forms of tissue storage iron on this relaxation rate. In patients with iron overload, almost all the excess iron is sequestered within cells as short-term storage iron in ferritin (soluble nanometer-sized particles, dispersed and relatively uniformly distributed) and as long-term storage iron in hemosiderin (insoluble and aggregated as irregular micron-sized clusters within siderosomes) (12–13). The differences in solubility and intracellular distribution between ferritin and hemosiderin iron produce distinct effects on MR signal decay. Dispersed, soluble ferritin iron affects signal decay principally through molecular spin-spin relaxation mechanisms (14–15), while aggregated, insoluble hemosiderin iron primarily induces magnetic field inhomogeneities and causes spin dephasing through susceptibility effects (16–17). Because the microscopic magnetic field inhomogeneities produced by aggregated iron are very efficient in causing spin dephasing at field levels of 1.5T and above, such conventional relaxation rates, in particular R2*, are predominately determined by hemosiderin iron and will not, in general, accurately reflect the ferritin iron level.

Cellular ferritin iron is in close equilibrium with the low-molecular-weight cytosolic iron pool (18–19) that is involved in cellular injury and eventually organ failure (20) and is accessible to iron chelators (19). Consequently, measurement of myocardial ferritin iron may be valuable in assessing the risk of iron toxicity in the heart and in monitoring the effects of iron-chelating agents. Recently, a new MRI approach has been developed (21) and validated (22–25) for separately quantifying ferritin and hemosiderin iron. This method exploits the property that aggregated hemosiderin iron can induce non-monoexponential multi-echo spin-echo signal decay (17,26). To evaluate the sensitivity of this new approach, we examined and compared RR2 with R2* and R2 in detecting myocardial iron changes associated with a brief suspension and resumption of iron-chelating therapy in transfusion-dependent thalassemia patients. We carried out cardiac MR examinations at 3T immediately before discontinuing iron chelation for 1 week, after a 1-week suspension of chelation, and after a 1-week resumption of iron chelation. We found that RR2 could detect differences in myocardial ferritin iron after as little as 1 week of changes in iron-chelating therapy.

Materials and Methods

Theory

In brief, non-monoexponential signal decay in the presence of dispersed ferritin iron and aggregated hemosiderin iron is predicted to approximately follow (21–22):

$$S(TE) = S_0 \cdot \exp(-RR2 \times TE) \cdot \exp(-A^{3/4} \times (\Delta t)^{3/4} \times TE^{3/8}), \quad \text{Eq. [1]}$$

where $S(TE)$ is the signal amplitude at echo time TE , S_0 is the initial signal amplitude, $2 \Delta t$ is the interecho time, $RR2$ is the reduced transverse relaxation rate, and A is the aggregation index. In tissues loaded with both ferritin and hemosiderin iron, $RR2$ is primarily sensitive to ferritin iron while A is predominately sensitive to hemosiderin iron (21–22). It is worth noting that in the absence of hemosiderin iron ($A = 0$), the signal decay is monoexponential and independent on the interecho time, and $RR2$ is simply equal to the conventional transverse relaxation rate, $R2$. This approach could provide an improved characterization of tissue storage iron and potentially lead to more accurate estimates of the total storage iron concentration (21–22).

Patients and 1-week Suspension of Iron Chelation

This study was performed in accordance with protocols approved by the Institutional Review Board. Patients with transfusion-dependent thalassemia ($N = 8$, 4 males and 4 females; mean age = 29.3 ± 8.6 years) receiving regular iron chelation were recruited, based on prior measurement of $T2^*$ ($= 21.9 \pm 3.1$ ms) at 1.5T. $T2^*$ of 20 ± 5 ms was chosen to be the selection criterion to ensure accurate measurement of $RR2$, $R2$ and $R2^*$ with sufficient signal-to-noise ratio and curve-fitting quality at 3T. Note that the clinical definition of cardiac iron overload is $T2^* < 20$ ms at 1.5T (8). Six patients were being treated with combination chelation therapy consisting of deferoxamine, 30 to 50 mg/kg for 2 to 5 days weekly and deferiprone, 55 to 95 mg/kg daily; two were being treated with deferiprone alone. Left ventricular ejection fraction measured at 1.5T was 65.1 ± 2.9 %, while serum ferritin level was 4475 ± 3100 pmol/L in these patients. Written informed consent was obtained from all subjects. Cardiac MR was performed immediately before discontinuing iron chelation for 1 week (Day 0), after a 1-week suspension of chelation (Day 7), and after a 1-week resumption of iron-chelating therapy (Day 14). This brief interruption of chelation therapy would likely lead to an increase of ferritin iron in myocardium due to its close equilibrium with the cytosolic iron pool, which would be expected to increase during the 1-week suspension of chelation. With resumption of iron-chelating therapy, the

myocardial cytosolic iron pool, and, in turn, the ferritin iron, would be expected to decrease back to levels near those observed on Day 0.

Cardiac MR

A single-breathhold ECG-triggered multi-echo fast spin-echo (FSE) sequence (27–29) was implemented to measure spin-echo signal decays on a 3T MRI scanner (Achieva, Philips Healthcare, Amsterdam, Netherlands) with maximum gradient strength of 40 mT/m and slew rate of 200T/m/s, and a 6-channel cardiac coil array for signal reception. An accelerated multi-echo spin-echo sequence with a turbo factor of 2, partial Fourier and sensitivity encoding (SENSE) acquisition, permitted single-slice multi-echo T2 mapping within a single end-expiratory breathhold. Specifically, two k-space lines were acquired per TR (= one cardiac cycle) for each effective TE, with odd echoes occupying the central k-space lines to minimize the first TE at high field (27). This multi-echo FSE sequence was previously used to measure T2 in a group of iron overloaded patients at 3T (27). In brief, one mid-ventricular short-axis slice with double-inversion black blood preparation was acquired with acquisition matrix = 128×96, turbo factor = 2, SENSE factor = 2, partial Fourier factor = 0.6, TR = 750–1200 ms, FOV = 370 mm, and slice thickness = 10 mm for 90° excitation (27,30) within a single end-expiratory breathhold (~15 cardiac cycles). Slice thickness of 30 mm was chosen for 180° refocusing in order to minimize stimulated echo effects (31). ECG trigger delay was set to the late diastole to minimize the effect of cardiac wall motion. Spin-echo signal decays with 3 different interecho times (5, 9 and 13 ms; 6 echo images each) were acquired to estimate RR2. Note that with turbo factor of 2 and odd echoes occupying the central k-space lines, the first effective TE was equal to the interecho time while the subsequent effective interecho spacing of echo images was twice the interecho time. The minimum first TE and shortest interecho time (i.e., 5 ms) were largely limited by the minimum duration of the selective 180° RF pulse, which was in turn limited by the maximum B1 of 13.5 μ T (associated with 25 kW peak RF power) for body RF transmission. To achieve this minimum TE, a 1.97 ms 90° self-refocusing excitation pulse was used together with a 1.52 ms 180° refocusing pulse. The total echo number (i.e., 6 effective echoes or echo images for interecho time of 5 ms) was limited by the maximum allowable specific absorption rate (SAR) and heart rates. Gradient crushers were applied around each refocusing pulse along all 3 directions with 0.68 ms duration and intra-voxel phase dispersion of 8.5π along each direction. For comparison, R2* measurement was performed in the same slice location using a single-breathhold ECG-triggered multi-echo gradient-echo (MEGE) sequence (32) with first TE = 1.55 ms, interecho time = 1 ms, echo number = 25, flip angle = 20°, turbo field echo factor = 4 and black-blood preparation (27,30) within a single end-expiratory breathhold (~10 cardiac cycles). Prior to R2 and R2* scans, B0 shimming was performed in a 3D volume covering the whole heart region. To minimize inter-subject and intra-subject variation, all subjects were trained for the end-expiratory breathhold procedure before MRI data acquisition. To improve measurement accuracy, the acquisitions described above were repeated five times at the same slice location during each exam for each subject.

Image and Statistical Analysis

Image analysis was performed using customized analysis software developed in MATLAB (Mathworks, Natick, MA). A region of interest (ROI) was placed within the interventricular septum to minimize the effect of susceptibility differences from the heart-lung interface (33–35). Identical ROIs in septum were used with slight position adjustments to account for motion among different breathholds (27,30). Spin-echo signal decays of the 3 different interecho times were simultaneously fitted to the non-monoexponential equation (Eq. [1]) with floating noise for RR2 measurement, i.e., 18 effective echo signals for estimating 4 unknown parameters. Specifically, nonlinear least square fitting was performed using

Levenberg-Marquardt algorithm, with signal intensity for shortest TE, 10 s^{-1} , $500 \text{ s}^{-3/2}$ and signal intensity for longest TE as initial values for S_0 , RR2, A and floating noise, respectively. The fitting performance of the Levenberg-Marquardt algorithm was tested with different initial values for RR2 and A, and considered stable because the estimated parameters converged to the same values. R2 was calculated using the spin-echo signal decay with the shortest interecho time (i.e. 5 ms) in which the magnetic field inhomogeneities and diffusion effects of hemosiderin iron were minimized. Both R2 and R2* were measured by monoexponential fitting of signal decays with floating noise. Note that the total acquisition periods of echoes or echo ranges were different for R2*, R2 and RR2 (26, 55 and 143 ms, respectively). Fitting with floating noise as an extra parameter was used to account for different noise levels. The five repeated trials or measurements were analyzed to obtain the average value for RR2, R2 and R2*. Repeated measures analysis of variance (ANOVA) with Tukey's multiple comparison test was employed to compare the RR2, R2 and R2* measurements among the three time points. Results were expressed as mean $\pm$ standard deviation (SD). A p -value of less than 0.05 was considered statistically significant.

Results

Figure 1 illustrates typical septum ROI delineation and the corresponding multi-echo FSE signal decays with interecho times of 5, 9 and 13 ms, demonstrating excellent reproducibility among 5 trials or breathhold measurements during a single MRI exam. The typical ROI size was $214 \pm 55 \text{ mm}^2$ in all patients at different time points. Note that the zigzag decay often observed by traditional Carr-Purcell Meiboom-Gill (CPMG) multi-echo spin-echo sequences was absent here because both odd echoes and the following even echoes were combined with the central k-space lines occupied by odd echoes. Figure 2 shows representative spin-echo signal decays in the septum ROI of different interecho times (5, 9 and 13 ms), indicating significant interecho time dependence and non-monoexponentiality of the signal decays.

Figure 3 shows the RR2, R2, and R2* changes in patients before (Day 0), and after chelation suspension (Day 7) and then 1 week after resuming iron chelation (Day 14). As compared with Day 0 ($19.8 \pm 5.6 \text{ s}^{-1}$), RR2 increased significantly ($p < 0.01$) at Day 7 ($22.1 \pm 5.4 \text{ s}^{-1}$); then decreased significantly ($p < 0.01$) at Day 14 ($20.0 \pm 5.6 \text{ s}^{-1}$). When compared to Day 0 ($34.5 \pm 10.7 \text{ s}^{-1}$), R2 increased ($p < 0.05$) at Day 7 ($37.0 \pm 12.8 \text{ s}^{-1}$); and then decreased ($p < 0.01$) at Day 14 ($33.5 \pm 11.1 \text{ s}^{-1}$). No significant differences in R2* were found between different time points ($76.5 \pm 35.9 \text{ s}^{-1}$, $74.1 \pm 39.0 \text{ s}^{-1}$ and $71.9 \pm 43.3 \text{ s}^{-1}$ at Day 0, 7 and 14, respectively). This finding indicates that transverse relaxation rates RR2 and R2, especially RR2, are more sensitive in detecting changes in myocardial iron. Between Day 0 and 7, the mean percent changes in RR2, R2 and R2* were $11.9 \pm 8.9 \%$, $5.4 \pm 7.7 \%$ and $-4.4 \pm 25.0 \%$, respectively. Between Day 7 and 14, the mean percent changes in RR2, R2 and R2* were $-10.6 \pm 13.9 \%$, $-8.9 \pm 8.0 \%$ and $-8.5 \pm 24.3 \%$, respectively. Note that no significant differences were found in R2 computed with other interecho times (i.e., 9 and 13 ms) as well as the average of three R2 values computed from 3 interecho times (data not shown).

Discussion

Within the cytoplasm of cells, metabolically active iron is present physiologically in low-molecular-weight forms destined for incorporation into functional compounds or, if present in amounts exceeding cellular requirements, for storage (36). Excess iron is normally first stored within the protein shell of ferritin, which is soluble and diffusely distributed within cells (12). As the amount of cellular iron to be stored increases further, the iron is gathered

within insoluble aggregates and clumps of varying sizes identified as hemosiderin (13). As the total amount of tissue iron increases, the proportion stored as hemosiderin rises, from trace amounts in normal individuals to 90% or more in patients with severe iron overload (37). Increases in the amount of cytosolic iron that exceed the cellular capacity for safe storage are believed to result in oxidative damage, ultimately leading to cell death, organ injury and failure (20). Recent studies have provided compelling evidence that iron entry and exit from ferritin are the result of an equilibrium based on the concentration of cytosolic iron (18–19), suggesting that ferritin iron may serve as indicator of cellular toxicity. Moreover, all three iron-chelating agents in clinical use, deferasirox, deferiprone and deferoxamine, decrease the intracellular concentration of ferritin iron, although by different mechanisms. Therefore, non-invasive measurement of cellular ferritin iron may provide early warning of iron-induced toxicity, and permit rapid monitoring of the effectiveness of iron-chelating regimes.

In the presence of both ferritin and hemosiderin iron, the analytic form of the signal decay, as shown in Eq. [1], can be regarded as a product of the monoexponential factor and a non-monoexponential factor that describes the more complex effects of diffusion in the spatially inhomogeneous field generated by aggregated hemosiderin iron (21–22). Most importantly, the effects of ferritin and hemosiderin iron on the signal decay are largely reflected in RR2 and A, respectively. The findings in the current study demonstrate the ability of RR2 to detect myocardial iron changes associated with a 1-week suspension of iron-chelating therapy in transfusion-dependent thalassemia patients. The current results also show that RR2 after resumption of chelation (Day 14) normalized to the baseline level (Day 0). Moreover, RR2 is shown to be more sensitive in detecting changes in myocardial iron than the commonly used relaxation rates including $R2^*$ and R2. These initial results suggest the feasibility of better characterization of myocardial storage iron, particularly ferritin iron, using the new transverse relaxation index RR2. This is likely a result of its superior sensitivity to soluble ferritin iron, which is in close equilibrium with the cytosolic iron pool that is expected to increase during the 1-week suspension of chelation and then decrease after resumption of iron-chelating therapy (18–19,38). Furthermore, along with the hemosiderin iron index A, RR2 approach may allow more accurate measurements of total tissue storage iron burden (21–22).

Clinically, determination of $T2^*$ ($= 1/R2^*$) in the interventricular septum has been used directly as an index of total myocardial storage iron (8,11,39). However, Anderson et al showed that myocardial $T2^*$, which is predominately sensitive to hemosiderin iron, changed only slowly after months of intensive iron-chelating therapy, despite significantly improved cardiac function within weeks (11). In line with this finding, no significant difference in $R2^*$ was found after suspending and resuming chelation therapy in the current study. Myocardial hemosiderin iron, as estimated by $R2^*$, would be expected to change little during the 1-week periods in our study. It should be noted that methods based on a single metric, such as $R2^*$ or R2, have an associated uncertainty reflecting variations in the relative amounts of ferritin and hemosiderin iron (40). In particular, these methods cannot accurately quantify ferritin iron levels in patients with high iron levels, in which ferritin iron can only be a small fraction of the total iron storage. It is noteworthy that because myocardial $R2^*$ increases prominently at 3T as compared with 1.5T, it is technically challenging to use $R2^*$ as an iron overload index at 3T where the shortest TE and interecho time can be limited by the peak RF power and gradient strength. More importantly, $R2^*$ measurement at high magnetic field ($> 1.5T$) is more vulnerable to increased B_0 inhomogeneity likely resulted from the increased susceptibility and shimming related effects.

With recent advances in the development of multi-echo FSE sequences that permit accurate acquisitions of multi-echo spin-echo signals in the heart during a single breathhold (27–29),

the RR2 measurement by acquiring spin-echo signal decays with three or more different interecho times has become feasible and practical with a clinically acceptable examination time. Given the specific interecho time dependence predicted by Eq. [1], use of two or more signal decays with different interecho times for fitting could improve the accuracy of RR2 measurement (22). Acquiring signal decays with three different interecho times as used in this study would be a reasonable compromise between accuracy and scan time for clinical applications. In addition, signal-to-noise ratio increase at 3T can facilitate the acceleration of breathhold acquisition sequence by use of large SENSE factor and half-Fourier sampling as demonstrated in the current study. Such reduction of breathhold time can be a major advantage in patient study, likely leading to more patient comfort and reliable measurements at 3T. On the other hand, it should be noted that for images with long echo times, signal-to-noise ratio could be compromised by increased R2 at 3T compared to 1.5T (34,41). Because R2* at high field increases substantially and its quantitation is vulnerable to increased B0 inhomogeneity, the rapid myocardial R2 and RR2 measurement protocols demonstrated in this study may constitute a robust alternative to the traditional R2* measurement for assessment of cardiac iron overload at 3T. Note that patient recruitment in this study was based on prior measurements of T2* ($= 21.9 \pm 3.1$ ms) at 1.5T with selection criterion (i.e., $T2^* = 20 \pm 5$ ms) to exclude patients with severe iron overload. This was to ensure sufficient signal-to-noise ratio in the signal decays at 3T, consequently improving the data and curve-fitting consistency and quality for RR2, R2 and R2* measurements. However, this selection criterion could also be a limitation of the current study because RR2 method could not be verified in patients outside this range of T2* (20 ± 5 ms at 1.5T). In addition, such selection criterion also limited the number of patients studied ($N = 8$) at our institution. Future study without T2* range restriction in a larger number of patients is warranted to further evaluate and validate the RR2 technique.

In conclusion, the experimental findings in this study demonstrate that RR2 measurement can detect myocardial iron changes associated with brief (1 week) changes in iron-chelating therapy in patients with transfusion-dependent thalassemia. While further studies in larger numbers of patients with wider ranges of iron loading over longer periods of time are needed, these initial results suggest that estimation of myocardial ferritin iron level by measuring RR2 may serve as a promising new means to rapidly evaluate the effectiveness of iron-chelating regimes in thalassemia patients. RR2 measurement may also allow monitoring the risk of iron-induced toxicity in patients with iron overload and be helpful in the evaluation of new candidate iron chelators.

Acknowledgments

Grant Sponsors: Hong Kong Research Grant Council: GRF7794/07M; Hong Kong Children Thalassaemia Foundation: 2007/02; National Institutes of Health: R01-DK069373, R01-DK066251, R37-DK049108, R01-DK049108; American Heart Association: 0730143N. This work was presented at the 2010 annual meeting of International Society of Magnetic Resonance in Medicine.

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Figure 1.

Typical ROI delineation in interventricular septum (**left**) and the corresponding multi-echo fast spin-echo (FSE) signal decays of repeated scans (**right**), demonstrating excellent reproducibility among 5 trials or breathhold measurements during a single MRI exam. Note that, with turbo factor of 2 and odd echoes occupying the central k-space lines, the first effective TE was equal the interecho time and while the subsequent effective interecho spacing of echo images was twice the actual interecho time. The signal intensities at the first TE in repeated measurements were normalized to unity for comparison.

Figure 2.

Representative spin-echo signal decays in the interventricular septum of different interecho times (5, 9 and 13 ms; 6 effective echoes each) in linear scale **(a)** and in logarithmic scale **(b)**, indicating significant interecho time dependence and non-monoexponentiality.

Figure 3.

Reduced transverse relaxation rate (RR2), R2 (using interecho time of 5 ms), and R2* values at Day 0 (on regular chelation), Day 7 (off chelation for 1 week), and Day 14 (on chelation again for 1 week). Repeated measures ANOVA with Tukey's multiple comparison test was performed with ** for $p < 0.01$, * for $p < 0.05$, n.s. for insignificance. DFO: deferoxamine; L1: deferiprone.