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Original Article

Thalamic paramagnetic iron by T2* relaxometry correlates with severity of multiple sclerosis

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Abstract

Iron can contribute to the pathogenesis and progression of multiple sclerosis (MS) due to its accumulation in the human brain. We focus on the thalamus as an information transmitter between various subcortical and cortical areas. Thalamic iron seems to follow different rules than iron in other deep gray matter structures and its relation to the clinical outcomes of MS is still indistinct. In our study, we investigated a connection between thalamic iron and patients' disability and course of the disease. The presence of paramagnetic substances in the tissues was tracked by T2* quantification. Twenty-eight subjects with definite MS and 15 age-matched healthy controls underwent MRI examination with a focus on gradient echo sequence. We observed a non-monotonous course of T2* values with age in healthy controls. Furthermore, T2* distribution in MS patients was significantly wider than that of age matched healthy volunteers ($P < 0.001$). A strong significant correlation was demonstrated between T2* distribution spread and the expanded disability status scale (EDSS) (left thalamus: $P < 0.00005$; right thalamus: $P < 0.005$), and multiple sclerosis severity scale (MSSS) (left thalamus: $P < 0.05$; right thalamus: $P < 0.005$). The paramagnetic iron distribution in the thalamus in MS was not uniform and this inhomogeneity may be considered as an indicator of thalamic neurodegeneration in MS.

Keywords: multiple sclerosis, thalamus, iron, relaxometry

Introduction

There is a broad consensus that multiple sclerosis (MS) is more than an inflammatory disease; it also involves some characteristic features of a classical neurodegenerative disorder. Besides cortical demyelination, MS involves progressive damage of various subcortical areas such as the basal ganglia, the hypothalamus, the spinal cord or the thalamus. Clinical progression varies greatly, reflecting complexity in

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pathophysiology. A method of quantifying disability in MS is the Kurtzke's expanded disability status scale (EDSS)[1]. The multiple sclerosis severity scale (MSSS) adds the element of disease duration to the EDSS and is designed to provide a measure of disease severity.

The research community believes that the ability to quantitatively and longitudinally assess regional brain iron has a potential role in the diagnosis of MS, as well as understanding pathogenesis, and monitoring disease progression and guiding treatment[2–3]. It still remains unclear whether iron deposition is simply an epiphenomenon resulting from brain tissue degeneration or if it directly contributes to brain damage in MS[4]. Iron plays a major role as a critical part of hemoglobin. Its deoxydated form deoxyhemoglobine and intracellular breakdown product methemoglobine are paramagnetic species producing local magnetic fields[5]. The forms of non-heme irons considered to be present in sufficient amounts to be detectable in MRI are ferritin and hemosiderin, which are found in various cell types[2,6]. Other non-heme iron forms, such as the free liable iron pool or Tf-bound iron, do not seem to influence MRI signal due to their low incidence[2,6]. In neurons, ferritin complex is assembled in the cell body and transported along axons. Ferritin that reaches its target area is degraded and iron can be utilized. However, this transport can be disrupted by axonal disruption which is caused by normal aging or disease processes and can lead to iron accumulation[4].

The use of T2* (or R2* = 1/T2* respectively) as a biomarker of iron is attractive due to its relative ease and efficiency of acquisition using a standard multi-echo, gradient echo sequence[7–11]. T2* is the value of combination of spin-spin interaction and magnetic field inhomogeneity and is more sensitive to microscopic magnetic field inhomogeneities than T2. In a postmortem study, R2* relaxometry was validated as an indicator of brain iron accumulation in a quantitative manner[12]. Khalil *et al.*[13] have recently demonstrated that R2* in the basal ganglia and thalamus was increased in MS patients compared with clinically isolated syndrome (CIS) and was also increased in MS compared with control subjects in the basal ganglia, but not in the thalamus. Furthermore, for the basal ganglia, but not for the thalamus, significant decrease of R2* level in CIS compared to healthy control was found. Using stepwise linear regression, subcortical R2* values were correlated with age, mental processing speed for global basal ganglia-the putamen, the pallidum and the caudate. The model excluded the variable disease duration and EDSS as well as the thalamus as a structure.

In our study, we focused on the thalamus as an information transmitter between various subcortical and cortical areas. It is a challenging part of the deep gray matter, where damage to the thalamus and its connections potentially impair a wide range of neurologic functions that may translate into significant cognitive, physical or mental disability. We chose T2* relaxometry as a possible tool for iron tracking. We sought to search for suitable tools that would be helpful in monitoring paramagnetic iron incidence and could be considered as an indicator of thalamic neurodegeneration in MS.

Patients and methods

Subjects

Twenty-eight subjects with definite MS (19 females, 9 males, mean age = 36 years, age range 22-57 years, mean disease duration = 8.25 years, range 1-22 years, mean EDSS = 3.44, range 1-8, mean MSSS = 4.95, range 1.13-9.74), and 15 age-matched healthy controls (10 females and 5 males, mean age = 39.3 years, age range 24-55 years) underwent MRI examination. The study protocol was approved by the local institutional review board at the authors' affiliated institution and informed written consent was obtained from all subjects.

Data acquisition

MRI experiments were conducted using an 8-channel volume head coil on a 1.5 T scanner (Magnetom Symphony, Siemens, Erlangen, Germany). T2* relaxometry was measured using the following parameters: gradient echoes, multi slice-sequences, interval between slices of 6 mm, slice thickness of 6 mm, pixel spacing of 2.34 mm × 2.34 mm, repetition time of 2,430 ms, echo times of 2.92, 7.73, 2.54, 17.35, 22.16, 26.97, 31.78, 36.59, 41.40, 50.00, 65.00, and 80.00 ms, echo number of 11, and flip angle of 78°.

Data analysis

Digital imaging and communications in medicine (DICOM) files from the scanner were read using bespoke software (developed in Matlab, Mathworks). Region of interest (ROI, ***Fig. 1***) was outlined manually. T2* values (ev. R2* = 1/T2*) were calculated separately by monoexponential fitting for each pixel outlined in the ROI. A standard used equation to describe the decay of relaxation signal S is:

$$S = noise + k \cdot \exp\left(\frac{-t}{T2^*}\right)$$

Where k was a particular constant for relaxation time $T2^*$, and t was the time when the signal was measured. Having once outlined ROI, we evaluated T2* and R2*

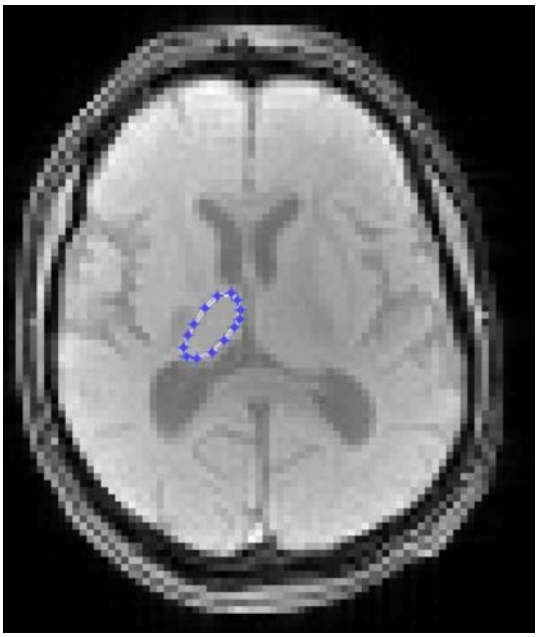

Fig. 1 **A raw data TE/RT 2.92/2460 ms, flip angle 78°, outlined thalamic region of interest (ROI).**

values from the same region. Common for all data sets, statistical analysis was carried out using the *t*-test with $P < 0.05$ considered significant.

Results

The calculated T2* values ranged from 59.8 ms to 72.4 ms [mean 69.3 ms (the left thalamus); 68.6 ms (the right thalamus)] and R2* ranged from 0.0137 ms^{-1} to 0.0167 ms^{-1} [mean 0.0144 ms^{-1} (the left thalamus) and 0.0145 ms^{-1} (the right thalamus)].

In healthy aging subjects, the quantity of iron in the thalamus was inverse peak shaped with the nadir after at the age of 35 (***Fig. 2***).

T2* distribution in MS patients was significantly wider than that in age matched healthy volunteers with $P < 0.001$ (age- and gender-matched MS *vs.* healthy subjects, evaluated for 15 available pairs). Moreover, T2* distribution spread was in a strong correlation with patients' parameters such as disease duration, EDSS and MSSS (***Table 1***). T2* distribution spread was also well observed within an MS group (***Fig. 3***).

Discussion

The exact underlying pathophysical mechanism leading to iron deposition in the deep gray matter, including the thalamus and the implications of this finding is still the matter of debate. In our study as well as in already published studies dealing with R2* relaxometry in MS, no significant correlation of thalamic T2*with EDSS, disease duration or MSSS was found. Although the thalamic iron is increased overall in patients with MS compared with that in control subjects, Walsh *et al.*[15] demonstrated a negative correlation of iron measured in the thalamus to MSSS in the multiple R2* regression, iron efflux. The suggested behavior of thalamic iron in the course of disease is not rare. In healthy aging, the thalamus iron quantity with age is suggested to be peak shaped with maximum at the age of about 30[16]. We confirmed these suggestions for the thalamus by obtaining peak shaped T2* against age in healthy volunteers. Consistently, the correlation of T2* with age over the whole age range in healthy volunteers was neither expected nor observed.

T2* distribution in MS patients was found signifi-

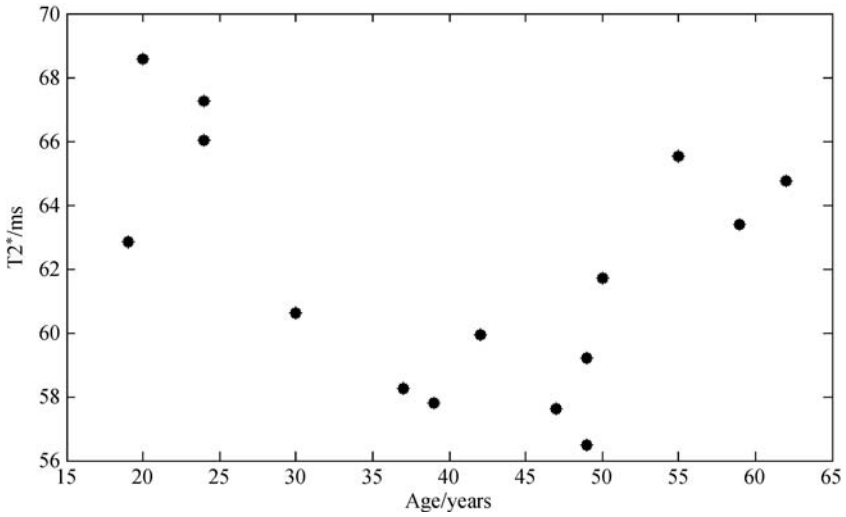

Fig. 2 **Healthy volunteers: inverse peak-shaped curve T2*** *vs.* **age, the left thalamus.**

***Table 1* *P*-values (*t*-test) of the correlation among T2* distribution parameters**

P-value	left thalamus			right thalamus		
	dd	EDSS	MSSS	dd	EDSS	MSSS
T2* mean / ms	-	-	-	-	-	-
T2* std / ms	$P<0.05$	$P<0.00005$	$P<0.05$	-	$P<0.005$	$P<0.005$

Note: mean and standard deviation (std) of the left and right thalamus with patients' parameters, dd = disease duration in years.

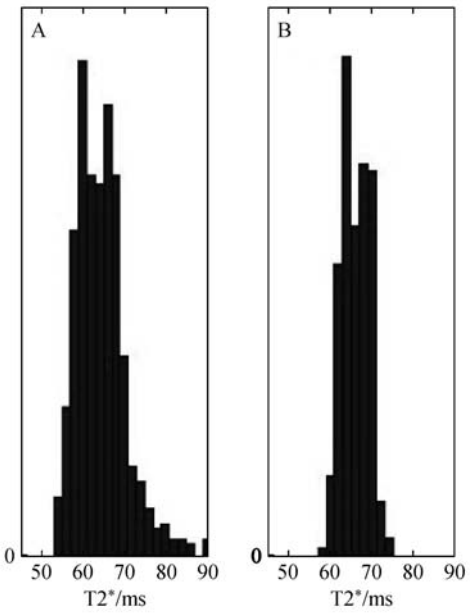

***Fig. 3* Distribution of T2* values in the left thalamus in two female patients with multiple sclerosis.** (A) EDSS = 3.5 age 22 years, and (B) EDSS = 1, age 22 years. Original images are enlarged by bicubical interpolation from 128×128 to 624×624 pixels to better visualize the T2* distribution.

cantly wider than that of age and gender matched healthy volunteers. This change is visible in T2* histograms and verifiable statistically. Considering standard deviation as a parameter expressing the width of distribution, we obtained the *P*-value of a pair test lower than 0.001. T2* distribution spread was also well observable within an MS group. It seems that the observed spread occurred primarily (i) due to increase of shortened T2* values, what is consistent with an iron accumulation in tissue and also (ii) due to increase of prolonged T2* at the other end of the spectrum, what is consistent with a reduced incidence of paramagnetic species. It indicates that in the course of MS, places with higher and lower iron content emerge. Shortened T2* appeared with increasing EDSS with various intensity. It means that there was not a uniform shift of T2* to lower values. This observation is coherent with those done in earlier studies, where brain iron was estimated from visual interpretation of R2* maps of the thalamus in MS[14,17].

In MS patients, T2* distribution spread correlated strongly with patients' disability (projected in EDSS), and with disease progress parameter MSSS (***Table 1***). The significant correlation with disease duration was found only in left thalamus. The fact that the disease duration predicts the iron distribution spread only moderately is not surprising. Disease duration is not easy to define due to the unknown period of asymptomatic disease evolution and often oligosympatic first episodes of MS. Since MSSS parameter is evaluated from known EDSS and disease duration, these parameters as well as their correlations are not independent.

In healthy subjects, the significant correlation of T2* distribution spread with age was found in the left and right thalamus (both $P<0.005$). For MS subjects, no significant correlation of the above mentioned parameter with patients' age was found. MS pathology that accumulates on the background of natural aging seems to have a primary effect on T2* distribution in the thalamus.

As our study showed, the non-monotonous course of T2* during aging and the exactly unknown behavior of T2* in the course of MS disease translates into wider T2* distribution in the thalamus. The thalamic iron concentration is not homogeneous over the whole tissue and the non-homogeneity increases in the course of disease with EDSS, and also with MSSS. We suggest the following situation: iron deposits resulting from interrupted axons or other damaged nerve cells cause T2* decrease. Additionally, the hypoperfusion of the thalamus in MS[18] with varying degrees of stasis in the capillary system causes a reduced incidence of deoxyhemoglobin, which results in T2* increase. The combination of both may finally result in the spreading of T2* distribution.

There were some drawbacks in our study. The use of the standard deviation as a parameter describing the distribution width is appropriate mainly for ideal normal distribution. As the obtained T2* distributions did not underlie any well known distribution ideally, but were approximable to normal distribution, we chose these values as a value easily available and understandable parameter. We should mention that T2* value and its corresponding transverse relaxation time R2* were criticized as an iron non-related MR estimation due to

other local background sources of magnetic field variation that cause signal loss unrelated to internal iron content[2]. Nevertheless, a number of previous studies using this MR parameter have obtained convincing results[19–21]. T2* and R2* are values that vary depending on proper field shimming, and the homogeneity of the external magnetic field is one of the critical parts of T2* relaxometry.

In conclusion, we observed and statistically evaluated non-uniform iron deposition in the thalamus in MS patients. This inhomogeneity in iron distribution was significantly increased with patients' parameters such as EDSS and also with MSSS in both thalamic structures and with disease duration in the left thalamus. The observed spread could be considered as an indicator of thalamic neurodegeneration in MS.

Acknowledgements

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