In Vivo Tissue Characterization Using Magnetic Techniques

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ABSTRACT

Among the few non-invasive methods to quantify liver iron deposits, magnetic resonance imaging (MRI) and biomagnetic liver susceptometry (BLS) have been considered the best to evaluate iron overload in the body. This diagnosis is necessary for patients who regularly receive red blood cells transfusion and that have a genetic disorder known as hemochromatosis. In this work, we present the evaluation of the clinical usefulness of MRI and BLS of hepatic tissue to quantify iron deposits in non-transfused and transfused patients. Liver iron evaluation by MRI and BLS were performed in a group of 48 patients. The MRI images weighted in T2 were acquired using multi-slice single-spin-echo (SSE) and single-slice multi-spin-echo (MSE), conducted on a 1.5 T whole body scanner. BLS measurements were performed using an ac superconducting susceptometer based on SQUID. Typically MRI is able to evaluate iron overload in liver as high as 30 mg/g dry tissue when using MRI scanners provided with specially designed pulse sequences. For higher iron concentrations susceptibility measurement works better than MRI to evaluate higher iron overloads in the liver, because in this case there is saturation of MRI signal.

KEY WORDS

Susceptometry, Magnetic resonance, Hemochromatose, Iron overload, SQUID.

INTRODUCTION

Iron overload in the body may occur secondary from increased gastrointestinal absorption or from repeated blood transfusions [Olivieri, 1994]. When chronically transfused and inadequately chelated patients develop cellular injury, skin hyperpigmentation and growth fails. This is followed in adolescence by pubertal failure, insulin-dependent diabetes, hypothyroidism, cardiac failure and arrhythmias [Fosburg, 1990] [Davis, 2000]. Practical management of iron overload requires a reliable estimation of body iron content and distribution, as well as understanding how iron overload translates into clinical consequences [Brittenham, 2001].

The most used method to evaluate total amount of body iron, in the majority of the clinical centers, is the measurement of the serum ferritin concentration in the blood. However, the correlation between serum ferritin and body iron is not sufficiently precise to be of strong prognostic value, mainly in situations of inflammation or tissue damage. Serum ferritin is also influenced by chelation and vitamin C treatment in a complex relationship with body iron content. Moreover, the relationship between serum ferritin and body iron appears to be different for different haematological conditions [Angelucci, 2002]. Another alternative to evaluate body iron overload consists in the measurement of liver iron concentration (LIC). Liver is the main iron storage in the body, containing approximately 70% of the total content [Angelucci, 2000]. Liver iron correlates closely with total body iron and can be assessed by biopsy or, more recently, by non-
invasive magnetic resonance imaging (MRI) and biomagnetic liver susceptibility (BLS). Although liver iron is more commonly investigated to estimate iron overload in the body due to the large size of this organ and its easy assessment, heart iron overload is the main cause of death in most transfused patients. So its measurement is very useful to guide the control of chelation therapy to avoid heart failure. Recently, it was observed that it is possible to estimate the heart iron level in relation to LIC by MRI image [Anderson, 2001].

MATERIALS AND METHODS

Subjects:

A group of 27 patients was studied (23 men, 4 women; age range, 7 - 68 years; mean age, 30 years), 13 had chronic anemia, and receive blood transfusions regularly, 1 had hemochromatosis, 13 had hepatitis C. Only 17 patients of this group have the heart iron store evaluated by MRI, because in the beginning of the research we didn’t acquire image of this organ. The Ethics Committee of the Hospital and Clinics of Faculdade de Medicina de Ribeirao Preto approved the study protocol and informed consent was obtained from all the patients.

BLS procedure for body iron assessment:

The biomagnetic liver susceptometry (BLS) was evaluated using a susceptometer based on a RF-SQUID (Superconducting Quantum Interference Device). This system uses a homogenous (5ppm variation in 10 cm along the Z direction) AC magnetizing field with low intensity and low frequency (114 µT and 7.7 Hz) applied on the hepatic region. The field detector consists of a second order gradiometer coil with 2.5 cm diameter and 4 cm baseline [Carneiro, 2003]. A water bag of approximately 5 liters surrounding the torso was used to minimize other tissues’ contribution. The water bag was used together with a special vacuum mattress that also immobilizes the subject. The BLS system with a patient positioned is illustrated in Fig. 1. An ultrasound image was made to evaluate the depth and size of the liver, and the distance between the liver and the lung. These measurements were made with the patient dressed in a non-magnetic gown lying down in the position of the susceptometric measurement (supine position with the body rotated about 35° relative to vertical axis). During the measurement, the bed moved down 8 cm with a velocity of 2 mm/s.

The magnetic flux variation on the BLS coil sensor is directly proportional to the iron store distributed inside the liver tissue. From the BLS signal ($\Delta V_{\text{BLS}}$) the liver iron concentration LIC is calculated using a linear fit with the following equation:

$$\Delta V_{\text{BLS}} = C \times (a \times \text{LIC} + b)$$

where $C$ is the calibration factor; $a$ is an angular factor that depends on the mass susceptibility of iron ($1.6 \times 10^6$ m$^3$/Kg) on the liver volume and on the vacuum permeability ($1.26 \times 10^{-6}$ H/m); $b$ is a linear coefficient factor that depends on the volume susceptibility of the air ($0.36 \times 10^{-6}$, inside the lungs) and of the tissue ($-9.0 \times 10^{-6}$), on the torso and lung volumes, and on the system (bed and water bag) contribution.

The calibration factor of the system is obtained making susceptibility measurement on a cylindrical phantom (diameter=20 cm, length = 30 cm) filled with pure water. The system contribution ($V_{\text{system}}$) is obtained making the measurement without the subject. The amplitude of $V_{\text{system}}$ is equivalent to the signal amplitude measured on a normal subject.
MRI procedure for iron assessment:

MRI images to evaluate liver iron overload were acquired using two different spin-echo (SE) sequences, conducted on a 1.5 T whole body scanner (Siemens Magnetom Vision Plus). The SE sequences were: a) a multi-slice single spin echo (SSE) (TR = 2500 ms; TE = 6, 7, 8, 9, 12, 15, 18 ms; slice thickness = 5 mm). b) a single slice multi-spin echo (MSE) with (TR = 2000 ms; TE = 22.5, 45,…,300 ms; slice thickness = 8 mm). The images using SSE sequence were reconstructed in a 192x256 matrix. For each TE, 19 slices were acquired covering all liver and spleen, and part of the heart. The seven repeated sequences were acquired under a fixed gain. It takes approximately four minutes to run the SSE sequence for each TE.

This SSE sequence with short TE was specially developed by Siemens and collaborators to evaluate liver iron overload using transverse relaxation evaluation. Only one slice in the middle of the major lobule was obtained using MSE sequence. They were reconstructed in 256x256 matrix and last approximately 5 minutes. One liter of saline solution was placed on the left side of the torso, as was indicated by Clark et al, to be used as intensity signal reference [Clark, 2000]. If no paramagnetic particles are present on the sample, the intensity amplitude should be the same for different TE, and therefore, the signal amplitude from the saline bag can be used as a standard. These SE sequences were performed without respiratory gating or breath-holding techniques.

As the TR value (2500 and 2000 ms) used in this measurement was significantly large when compared to typical T2 and T1 relaxation time of the liver tissue (>40 ms), the transverse relaxation rate $R_2 (=1/T2)$ was calculated fitting the image signal intensities by the following mono-exponential equation:

$$S_{SE}(TE) = S_{SE}(0)e^{-R_2TE} + S_{LO},$$

where $S_{LO}$ is the signal level offset and $S_{SE}(0)$ is the signal intensity at TE=0 ms.
The transverse relaxation rate, R2, was evaluated pixel by pixel on a selected region of interest (ROI), and a histogram was used to assess R2 distribution. A set of Gaussian functions was fitted to the R2 distribution and the weighted average value of the distribution that corresponds to the hepatic tissue was used as the R2 value. Fluctuations in voxel intensity, caused mainly by respiratory motion, were filtered using a 5x5 smoothing window, progressively centered on each voxel in the ROI. Variations of liver signal intensity (SI\textsubscript{liver}) between the images acquired with different TE were corrected using the signal intensity of the water bag imaged together with the patient (SI\textsubscript{water bag}). For the pulse parameters used, it was expected that the signal intensity coming from the water in the saline would have almost the same intensity for all TE. The selected ROI covered all liver area and attention was aimed to avoid vein regions. These same procedures were applied to evaluate relaxation rate R2 at spleen and heart tissue. For this last organ, the ROI was drawn on the left ventricular myocardium. Figure 2 shows the MRI images of these three organs with the ROI superimposed.

**RESULTS**

**Liver Iron quantification:**

When calibrating a new measurement method it is desirable to rely on a primary standard. If this is not available, a secondary standard can also be employed. For BLS atomic mass spectrometry is considered the gold standard or the primary standard to be compared, although as shown in the images above, the iron is not evenly distributed in the liver and several samples should be harvested in order to get a reliable measurement, a procedure that has critical ethical issues. This procedure was done by St Pierre et al. [St Pierre, 2004] when calibrating the relaxometry method by relating the R2 to liver iron concentration obtained by chemical assay in 105 liver biopsies. In our case the iron concentration in phantoms and subjects in a wide range of concentration were first evaluated by MRI relaxometry, using the same SSE sequence and MRI scanner, and then by BLS thus generating a calibration curve for BLS. In metrological terms BLS was referred to a secondary standard. Thus, images from 23 patients and of a paramagnetic phantom with different concentrations of MnCl were acquired.

The BLS results were well correlated with LIC estimated by SSE sequences using St Pierre’s protocol (0.93). The R2 value calculated from MSE images presented a poor correlation with the LIC by BLS. The main cause of this result was the long TE used to acquire the images. Figure 3 shows the relation between LIC by BLS and by MRI with SSE sequence and mean logarithm of intensity for the first echo.
(TE=22.5ms) acquired with MSE sequence. A higher correlation (0.96) was found to BLS results. In an attempt to verify the possible association between iron in different deposits, the signals from liver, spleen and heart were compared. No correlation was observed between the R2 value from the liver tissue versus the R2 from the spleen tissue. But, as shown on Fig. 4, a low correlation (0.73) was found between R2 values from the liver tissue versus R2 from the heart tissue. It suggests that the measurement of the liver concentration can be an indicator of the iron present in the heart.

**DISCUSSION**

The iron concentration determined with BLS using a secondary standard calibration were in accordance with the clinical symptoms. The BLS procedure avoids the biopsy and all the possible related complications in a group of patients already with severe conditions.

The present work confirms the feasibility of using two non-invasive methodologies to quantify liver iron concentration from normal to the highest levels found in clinical diagnostic. The methodology presented has potential application to follow and guide the control of the tissue iron balance in patients submitted to blood transfusion and iron chelation treatment, including the need to evaluate the efficacy of new oral chelation agents. The use of MRI to evaluate heart iron level is another tool to be used to control chelation of regularly transfused patients. Due to difficulty to find a calibrated curve of T2 versus iron content on the heart, once that it isn’t indicated to make biopsy of this organ, we suggest an indication of heart iron concentration (HIC) relative to LIC. It is possible, when the MRI measurement is realized by a MRI protocol calibrated to liver iron quantification as St. Pierre’s one. This way, it is easier to follow those cases where the HIC is bigger than LIC. The low correlation (73%) observed here between the R2 of the heart and liver tissue of regularly transfused patients show that heart iron loading presents an individual
variance. We can see from Fig. 4 that there are cases where the LIC evaluated by BLS and MRI is high, but the iron level on the heart is low.

This work presented a study of tissue iron overloading using susceptibility and relaxation time measurement obtained from different machines and different procedures. With the techniques of performing MRI with SQUIDs at very low magnetic fields being developed at present, the possibility of simultaneous relaxation and susceptibility measurements seems an interesting perspective to advance our knowledge of iron overload mechanisms [Lee, 2005].

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REFERENCE


