Susceptibility Mapping in Sickle Cell Anaemia Patients With and Without Chronic Blood Transfusions

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INTRODUCTION: Sickle cell disease is a group of haemoglobinopathies involving a point mutation in the β-haemoglobin gene. The most common and severe form is homozygous sickle cell anaemia (SCA). In the UK, SCA affects 1 in 2000 births[1]. Patients with SCA have increased risk of stroke and regular blood transfusions are commonplace to decrease stroke risk. Transfused patients are known to have iron overload, particularly in the liver[2] and may receive iron chelation therapy. Tissue magnetic susceptibility (χ) derived by MRI susceptibility mapping has been shown to correlate strongly with tissue iron content[3-5] and one study of transfused SCA patients[6] has shown increased χ in some iron-rich deep-brain gray-matter regions. Therefore, we mapped the χ in SCA patients transfused (for reasons other than to prevent recurrent stroke) and healthy controls to test the **hypothesis H1: that iron content in deep-brain regions is higher in transfused SCA patients than in healthy controls**. The iron content of deep-brain regions in non-transfused SCA patients has not been studied so we mapped χ in a large cohort of non-transfused SCA patients and healthy controls. SCA patients have a lower haematocrit than healthy controls and are not orally supplemented with iron, therefore **hypothesis H2: is that the iron content in deep-brain regions is lower in non-transfused SCA patients than in healthy controls.** Purpose: It is clinically important to measure brain tissue iron content in SCA patients as this could inform their treatment in future.

METHODS: Cohort: 25 children and adults with SCA showing no evidence of cerebral infarction on T2-W MRI and 18 matched controls. Subjects with motion artifacts were excluded. Pilot study: To test **H1** and **H2** we selected all 5 patients treated with regular blood transfusions for 2-10 (median 5) years for severe disease e.g. frequent pain. We also included 5 age-, gender- and race-matched SCA patients not on any treatment and 5 similarly matched healthy controls. The mean/(range) of ages were 18.3/(10.9-28.4), 16.8/(10.1-29.4) and 17.8/(9.6-27.8) for transfused, healthy and non-transfused groups respectively. Each group of 5 had 2 females. Larger study: **H2** was tested further in 20 non-transfused SCA patients (11F) and 18 controls (9F). The groups had very similar age distributions: mean/SD = 15.3/6.6 v. 14.8/5.7 in patients v. controls. MRI Acquisitions: Images were acquired on a clinical 1.5 T Siemens system with a 32-channel RF coil. The 3D gradient echo sequence had parameters: TR = 40 ms, $\alpha = 25°$, 5 echoes: TE1 = 4.94 ms, ΔTE = 7.35 ms, FoV = 256 x 176 x 120 mm, 1 mm isotropic resolution, GRAPPA acceleration x 3 in the PE direction with 24 reference lines, 7/8 partial Fourier acquisition in both PE directions, BW = 140 Hz/pix and total acquisition time = 5 mins 44 s. A T1-W image was also acquired using a 3D FLASH sequence (TR = 11ms, TE= 4.94ms, $\alpha = 15°$, 1 mm isotropic resolution) to allow automated region-of-interest (ROI) segmentation. Image Processing: For each subject, complex images from all 5 TEs were fitted[7] to generate a ΔB map. Residual phase wraps and some background fields were removed by Laplacian unwrapping[8] using a FSL BET brain mask (no. erodes = 3, TSVD threshold = 0.02). Residual background fields were removed using SHARP[4] with a spherical kernel radius = 1 mm at the edge of the brain to 20 mm at the centre[9]. χ mapping used a TKD technique[3] (t = 1.5 to minimise streaking artifacts) and underestimation of χ was corrected by dividing by 0.35[8]. R2* maps were calculated by a linear fit of log(magnitude) values over all 5 TEs as a reference. ROI Analysis and Statistics: The T1-W image was co-registered to the T2*-W magnitude image using FSL FLIRT. FSL FIRST was applied to the co-registered T1-W images to segment left and right caudate (CN), putamen (PT) and globus pallidus (GP) ROIs for each subject (e.g. see **Fig 1**). The ROIs were applied to yield mean χ and R2* for each ROI in each subject. For each study, ANCOVA statistics were used to investigate differences in ROI mean χ and R2* values between groups, controlling for age, gender and any interaction between age and gender. Post-hoc t-tests were used to investigate significant differences between groups.

RESULTS: An example χ map from a non-transfused SCA patient is shown in Fig. 2. The GP is bright as it has high iron content. Pilot study: Table 1 shows the mean and standard deviation (SD) of the χ values in each group and ROI and ANCOVA results. There were no significant effects on χ of age or gender. There was a significantly lower χ in the GP in both SCA patient groups than in controls. An ANCOVA on the R2* values showed no significant differences by group or gender but showed highly significant positive correlations of R2* with age ($p < 0.018$). Larger study: Similar mean χ and R2* values were obtained. There was a trend for lower GP χ in SCA. The ANCOVA on the χ values showed no significant effects except for a significant positive correlation of CN χ with age ($p = 0.003$). An ANCOVA on the R2* values showed no significant effects of group or gender but showed highly significant positive correlations of R2* with age ($p < 0.00024$) as well as some significant effects of gender × age interactions.

Table 1. Pilot Study **ROI**	**Caudate**		**Globus Pallidus**		**Putamen**	
Susceptibility (x 10^{-2} ppm)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Healthy Control	1.95	0.52	6.50	1.17	-0.25	0.53
Non-Transfused SCA	1.39	0.59	4.46	1.14	0.11	0.46
Transfused SCA	1.98	0.50	4.69	1.01	-0.11	0.45
ANOVA F (p) – Group	2.28 (0.16)		6.68 ($0.02^{a,b}$)		1.30 (0.32)	

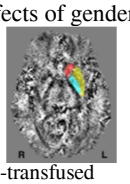

Figure 1. ROIs in the CN (red), PT (yellow) and GP (aqua) on the χ map of a transfused SCA patient.

t-tests: a: Controls > Non-transfused SCA ($p = 0.014$) **b**: Controls > Transfused SCA ($p = 0.015$)

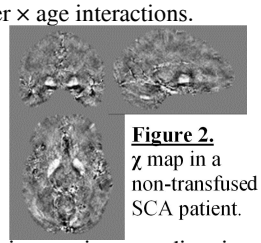

Figure 2. χ map in a non-transfused SCA patient.

DISCUSSION AND CONCLUSIONS: In this cohort, the ROI mean χ values are similar to but lower than in previous studies in haemoglobinopathy patients[6,10] probably because those studies measured only positive χ values within each ROI whereas all (both positive and negative) values were included here. The SD of ROI χ values is similar to previous studies[6,10]. The pilot study showed a significantly lower χ in the GP in both SCA patient groups than in controls. Transfused patients did not have high χ. This supports **H2** and agrees with the lower χ values found in the GP of β-thalassaemia patients compared with controls[10]. The lack of significant results in the larger study does not provide strong evidence to support **H2** although there was a trend to lower χ in the GP of SCA patients. An increase in the cohort size, including patients with infarction, would allow further investigation. Significant positive correlations were found between R2* values and age as expected[11]. The lack of significant positive correlations of χ with age[12] - except in the CN - is probably due to the relatively low SNR phase and χ maps. Several ΔB and χ maps showed an artifact due to open-ended fringe lines in the original phase images. This may be overcome by improved combination of the multiple RF coil data[13]. In future, we intend to investigate other ROIs including the choroid plexus, red nucleus and substantia nigra. The suggestion of decreased iron content in the GP in SCA patients is clinically important as, if proven, it may explain some cognitive deficits and affect patient treatment.

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