

Assessment of carotid plaque inflammation in diabetic and non-diabetic patients-an exploratory USPIO-enhanced MR imaging study

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Abstract:**Background:**

Ultrasmall superparamagnetic iron oxide (USPIO)-enhanced magnetic resonance (MR) imaging enables identification of inflammation within atheroma, predominantly by USPIO uptake by macrophages present in atherosclerotic tissue. Diabetic patients however may have dysfunctional macrophage activity, which may affect utilisation of USPIO in identifying plaque inflammation in this patient cohort.

Methods:

Fifteen diabetic and fifteen non-diabetic patients underwent USPIO enhanced-carotid MR imaging using 1.5T MR system. Pre and post-USPIO carotid MR images were manually co-registered. The percentage decrease in the signal intensity after USPIO administration was calculated as a relative measure of the USPIO uptake.

Results:

Diabetic and non-diabetic patients had comparable demographics and co-morbidities. The mean global, maximum quadrant and maximum slice changes showing change in relative signal intensity as a result of USPIO administration was comparable for the two patient cohorts ($p>0.05$).

Conclusions:

USPIO can identify inflammatory burden with carotid atheroma in both diabetic and non-diabetic patients.

Keywords: Atherosclerosis; USPIO; carotid; MRI; nanoparticles

Introduction

Inflammation plays an important role in determining vulnerability of atherosclerotic plaques¹. It has been observed that high-risk plaques have a higher inflammatory burden than stable atheroma. Identification of high-risk plaques and treatment strategies targeted at the underlying inflammatory process is therefore clinically beneficial, if the increasing morbidity and mortality associated with atherosclerosis is to be controlled. Ultrasmall superparamagnetic particles of iron oxide (USPIOs) have been successfully used in magnetic resonance (MR) imaging studies to investigate pathophysiology of atherosclerosis and assess feasibility of anti-atherosclerotic treatments².

Pathological analysis of plaque specimens has identified that USPIO can be isolated from macrophages within atheroma, indicating that USPIO uptake within the atheroma predominantly represents the macrophage activity and therefore the degree of plaque inflammation^{3, 4}. USPIO uptake appears as areas of low signal intensity on T₂*-weighted sequences due to superparamagnetic effects of the iron oxide particles. Other probable mechanisms of USPIO uptake in atheroma include: transcytosis of USPIOs through the dysfunctional endothelium complemented by various triggers (such as lipid retention, oxidative stress from free radical damage etc) or entry of USPIOs through leaky neovessels within atheroma².

Patients with diabetes are more prone to develop atherosclerosis as diabetes amplifies the entire atherosclerotic mechanism from endothelial cell dysfunction, oxidative stress, macrophage chemotaxis and adhesion to diapedesis⁵⁻⁷. Since the uptake of USPIOs seems to predominantly depend on the macrophage uptake because of its macrophage-selective properties, it can be hypothesized that its utility may differ in patients with defective macrophages such as in diabetics.

This study therefore aims to compare carotid plaque inflammation of diabetics and non-diabetic patients using USPIO-enhanced MR imaging.

Methods

The pooled patient cohorts from two former prospective studies^{8, 9} were used for this study. Approval from institutional ethics committee was obtained. All patients gave written informed consent before recruitment. Patients with clinically documented atherosclerotic carotid disease and who had demonstrated the presence of inflammation within their carotid lesions on USPIO-enhanced MRI regardless of symptomatic status were included in this study. Fifteen diabetic and fifteen non-diabetic patients were randomly selected from the patient pool.

Inclusion criteria included: patients reported with carotid atherosclerotic disease (either asymptomatic or previously symptomatic) with a minimum degree of stenosis of 40% on at least one side on the screening ultrasound examination were included in the study. Exclusion criteria included patients with any contraindication to MR imaging (e.g. pacemaker, metal

stents or implants), renal insufficiency, allergy to dextran or iron salts or history of malignancy were excluded.

MR Acquisition

Sinerem (Fermoxtran-10) in a dose of 2.6mg/kg was administered to the patients in the form of slow infusion, diluted in normal saline through an indwelling large-bore intravenous cannula over 30 minutes. The patients received baseline (pre-contrast) imaging and post contrast imaging after 36 hours of administration of USPIO infusion. All patients underwent the multicontrast imaging of both the carotid arteries using 1.5T whole body MRI system (GE Diagnostic Imaging, Waukesha, WI) and 4-channel phased array neck coil (PACC, Machnet BV, Elde, The Netherlands). Movement artefact was minimised using a dedicated vacuum-based head restraint system (VAC-LOK Cushion, Oncology Systems Ltd, UK) to maintain the head and neck in a comfortable position and aids in close approximation of the surface coils.

After an initial coronal localizer sequence, axial 2-dimensional time-of-flight MR angiography was performed to identify the location of the carotid bifurcation and the region of maximal stenosis on each side. Axial images were acquired through the common carotid artery 12 mm (4 slices) below the carotid bifurcation to a point 12 mm (4 slices) distal to the extent of the stenosis identified on the time-of-flight sequence. This method ensured that the entire carotid plaque was imaged and also facilitated image co-registration during repeat imaging.

The following 2-D electrocardiography (ECG)-triggered, fat-suppressed fast spin echo pulse sequences employing double-inversion recovery blood suppression with a voxel size of $0.4 \times 0.4 \times 3\text{mm}$ were used in each case: T₁-weighted (repetition time [TR]/effective echo time [Teff]/echo train length [ETL]: 1 R-R/7.8 ms/12), A quadruple inversion recovery 2-D ECG-triggered T₂*-weighted spiral sequence was performed with the following scan parameters: FOV 12cm, NEX 2.6ms, TR/effective echo time 1 R-R/5.6ms, flip angle 60°, slice thickness 3mm and no interslice spacing.

Image Analysis

The degree of luminal stenosis was assessed according to the European Carotid Surgery Trial (ECST) criteria. Pre and post-USPIO MR images were manually co-registered according to plaque morphology and distance from the carotid bifurcation at the time of imaging. Using the quadrant approach the vessel wall in each slice is divided into quadrants by drawing perpendicular lines to the horizontal axis across the image using OsiriX (version 5.2.2, Pixmeo, Bernex, Switherland). Signal intensities in each quadrant were normalised to the adjacent sternocleidomastoid muscle pre- and post-USPIO infusion. The percentage decrease in the mean signal intensity is defined as the relative measure of the uptake of USPIO.

Statistical Analysis

Continuous variables are presented as median (interquartile range-IQR). Data normality was assessed by Shapiro-Wilk's test. Unpaired t-tests were used for comparison of continuous variables with normal distribution. Chi-square test was used for comparison of portions. P-values <0.05 were defined as statistically significant.

Results

Thirty patients (23 males; 7 females) were included in the analysis. Patient demographics are presented in Table 1. The two clinical groups of diabetic and non-diabetic patients had comparable demographics and co-morbidities (Table 2). The signal void within a plaque due to USPIO uptake can be seen on QIR spiral T2* pulse sequence (Figure 1). The mean global, max quadrant and max slice changes showing change in relative signal intensity as a result of USPIO administration is reported in Table 3 and Figure 2. No statistically significant differences were noted for the DM and non-DM cohorts.

This suggests that USPIO is effective to identify inflammation within atheroma of diabetic and non-diabetic patients.

Table 1: Patient Demographics

Characteristics	n (%)
Number of patients	30
Male/ Female	23/7 (76%/23%)
Median age (yrs) [IQ]	72 [67-77}
Mean Luminal Stenosis (%) [IQ]	56.5 [46.4-69.0]
Diabetes	15 (50%)
Hypertension (HTN)	17 (56%)
Ischemic Heart Disease/ MI	12 (40%)
Vascular events (TIA)	20 (66%)
Previous CABG	6 (20%)

IQ: interquartiles, CABG: coronary artery bypass grafting, luminal stenosis assessed according to European Carotid Surgery Trial (ECST) criteria, MI: myocardial infarction, TIA: transient ischaemic attack.

Table 2: Demographics of diabetic and non-diabetic patients

	Diabetics (n=15)	Non-diabetics (n=15)	P value (two-tailed)
Age (yrs)	77 (69.5-78)	70 (62-73)	0.06 [^]
Luminal stenosis	58.72 (51.96-67.46)	51.9 (45.6-69.14)	0.84 [^]
Symptomatic (TIA/ non-disabling stroke)	10 (66%)	10 (66%)	1.00*
Ischemic Heart Disease	7 (46%)	5(33%)	0.70*
CABG	4 (26%)	2 (13%)	0.64*
HTN	12 (80%)	6 (40%)	0.06*

[^] unpaired t-test, *chi-square

Table 3: Ratios of relative difference signal intensity relative to the sternocleidomastiod pre- and post-USPIO uptake for diabetic and non-diabetic patients

	DM	Non-DM	p-value
Mean Change	12.3[5.9-18.1]	9.2[5.6-19.0]	0.831
Max Slice Change	21.3[13.4-30.5]	15.2[11.6-26.3]	0.591
Max Quadrant Change	37.9[25.6-45.1]	35.8[24.8-46.1]	0.978

median[IQ]

Discussion

The results of this study indicate that the carotid plaques of diabetic and non-diabetic patients have comparable inflammatory burden as indicated by non-significant difference between reductions of MR quantified-relative signal intensities following USPIO administration. It has already been validated by our group that these areas of signal void correspond to macrophage rich foci within carotid atheroma⁴. This implies that despite have dysfunctional white blood cells (including macrophages), MR identifiable-USPIO uptake is demonstrated in carotid atheroma of diabetic patients. The clinical utility of USPIO in assessing plaque inflammation is therefore not apparently limited by a patient's diabetic status. One explanation may be that despite having somewhat dysfunctional macrophages, other routes of USPIO uptake within atheroma may well compensate in diabetic patients i.e. transcytosis through arterial intima and/or leaky neovessels, thereby maintaining ability of this contrast medium to assess plaque inflammation.

Diabetes mellitus is a chronic metabolic disease characterised by increased blood glucose level (hyperglycaemia) either because of lack or resistance to insulin. Diabetics are more prone to develop complex ischaemic vascular disease because it exacerbates the atherosclerotic process. There is alteration in the regulatory mediators in the body that leads to endothelial cell dysfunction, defective glycosylation of extracellular matrix and inappropriate macrophage properties. Moreover immune system in diabetics is vastly impaired that increases the likelihood of developing atherosclerosis. It has been reported that polymorphonuclear neutrophils (PMNs) of diabetics exhibits reduced chemotaxis compared to the healthy individuals⁵. Most of the functions of PMN are energy-dependent, inadequate supply of glucose to the cells due to lack of insulin hinders the functions of PMNs such as chemotaxis¹⁰; however adhesion may or may not be affected⁵. Phagocytosis and chemotaxis of monocytes is markedly impaired especially in patients with poorly controlled diabetes. There is decreased synthesis of proinflammatory cytokines following lipopolysaccharide stimulation and also intrinsic defect in the monocytes themselves¹¹. The cellular response of monocytes to Vascular Endothelial Growth Factor-A (VEGF-A) also seems to be enervated in diabetic patients¹². Impairment in macrophage phagocytic activity and chemotaxis leads to suppressed cellular innate immunity, thereby increasing the chances of inflammation and infections.

As inflammation plays a crucial role in the progression of the vulnerable plaque, it is an emerging target in the management of atherosclerosis. USPIO-enhanced MR imaging has proved to be a beneficial *in vivo* marker of inflamed human atheromatous plaques. This is primarily because USPIOs alter MR relaxation times and the macrophage's susceptibility to phagocytose these iron oxide nanoparticles makes it a good macrophage-specific agent. Other routes of USPIO uptake may also play a role in assessing functional status of vulnerable plaques irrespective of the impaired immune system in patients with diabetes. All of the diabetic patients in this study had well-controlled diabetes mellitus. The efficacy of USPIO in poorly controlled diabetics may need further exploration. It is also noteworthy that the proportion of symptomatic and asymptomatic patients among the diabetic and non-diabetic patient cohort was also similar therefore it is unlikely to be a confounding factor while interpreting the above results.

Conclusions:

USPIO-enhanced carotid MR imaging can identify inflammatory activity of atherosclerosis in diabetic and non-diabetic patients. A histopathological study comparing USPIO uptake in plaques of both patient groups would help further validate our hypothesis.

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Figure Legends

Figure 1:

Panel 1: Pre-USPIO quadruple inversion recovery (QIR) 2D ECG triggered T_2^* -weighted spiral sequence image of carotid plaque of 'non-diabetic' patient (lumen-L), 1b shows post-USPIO QIR spiral image with signal drop out (signal void-indicated by black arrow) within the plaque (P).

Panel 2: Pre-USPIO (2a) QIR spiral sequence image of carotid plaque of 'diabetic patient' (lumen-L), 2b shows post USPIO QIR spiral image with signal drop out (signal void-indicated by black arrow) within the plaque (P).

Figure 2: USPIO update analysis at plaque (a), maximum slice (b) and maximum quadrant (c) show no discernable differences between the diabetic and non-diabetic cohorts

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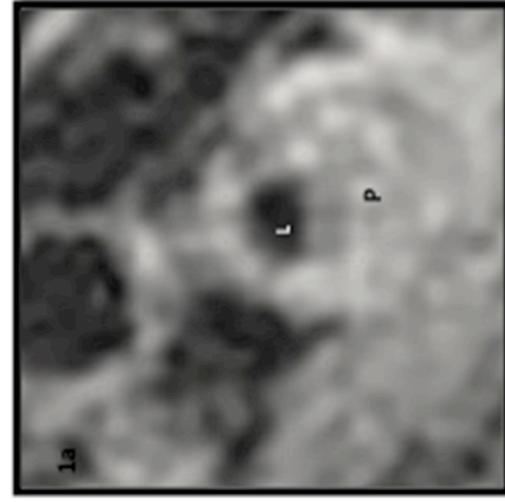
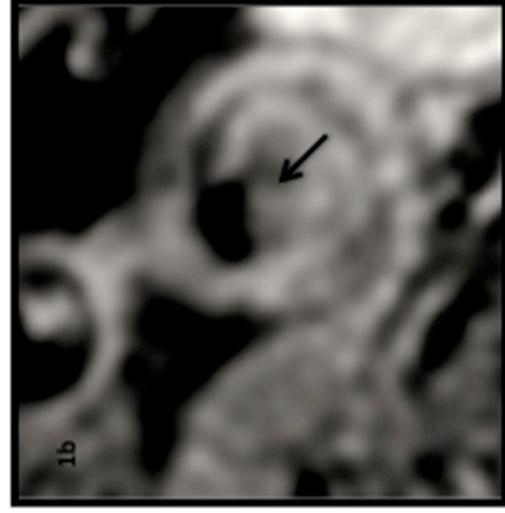
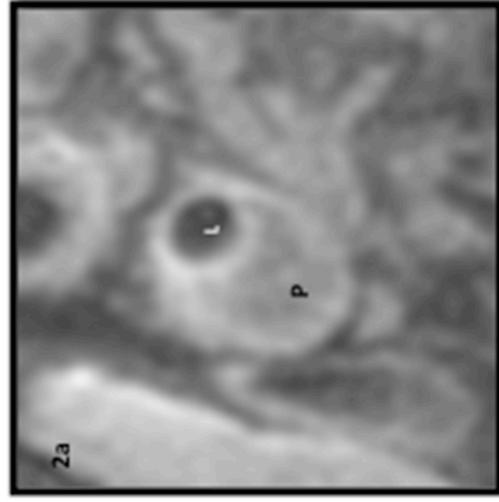
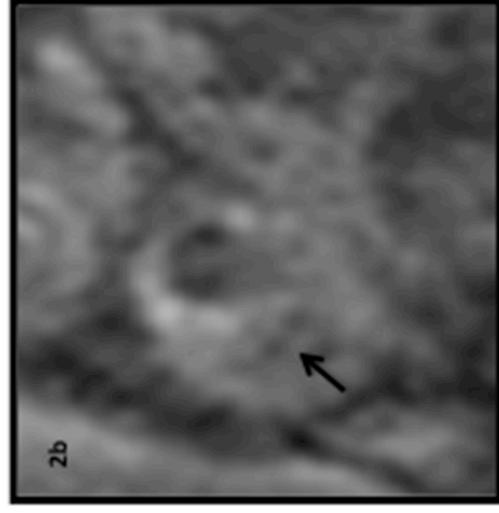


Figure 1:

Panel 1: Pre-USPIO (1a) quadruple inversion recovery (QIR) 2-D ECG-triggered T_2^* -weighted spiral sequence image of a carotid plaque of 'non-diabetic' patient (lumen-L), 1b shows post-USPIO QIR spiral image with signal drop out (signal void-indicated by black arrow) within the plaque (P).



Panel 2: Pre-USPIO (2a) QIR spiral sequence image of a carotid plaque of 'diabetic' patient (lumen-L), 2b shows post-USPIO QIR spiral image with signal drop out (signal void-indicated by black arrow) within the plaque (P).

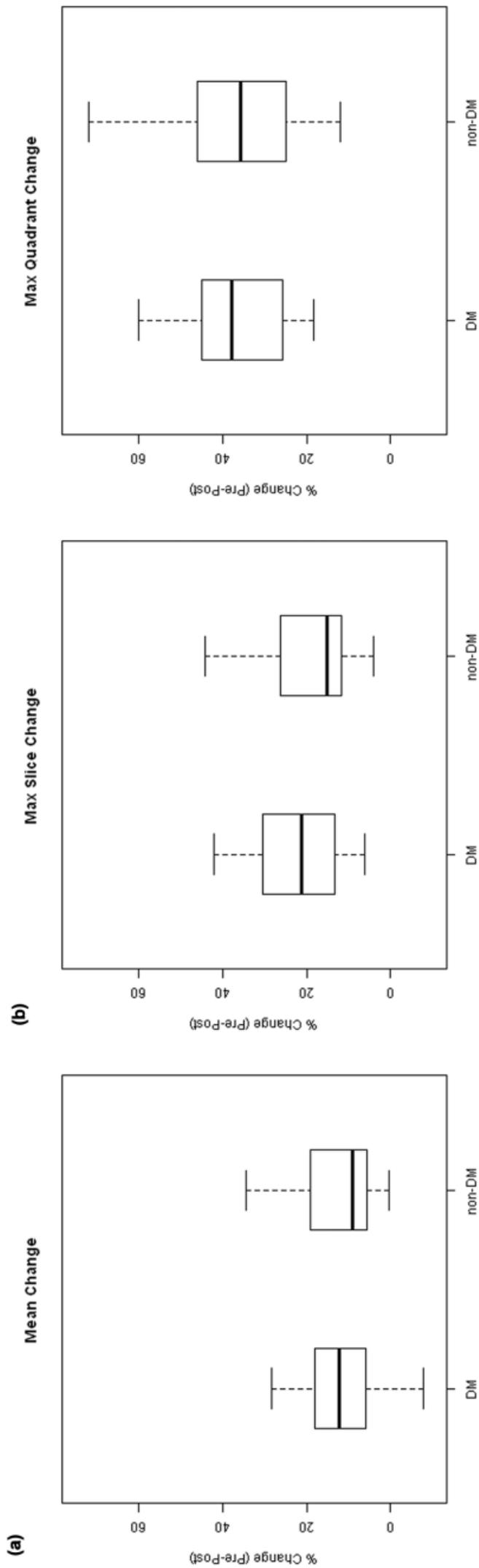


Figure 2 : USPIO uptake analysis at plaque (a), maximum slice (b) and maximum quadrant (c) show no discernable differences between the diabetic and non-diabetic cohorts