DIFFUSION TENSOR IMAGING OF THE ROTATOR CUFF MUSCLES: OPTIMISATION OF B VALUE AND NUMBER OF DIFFUSION DIRECTION FOR IMAGING AT 3-TESLA MRI

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Dissertation Submitted in Partial Fulfilment of the Requirements for Master of Medicine (Radiology)

UNIVERSITI SAINS MALAYSIA

2018

ACKNOWLEDGEMENT

The completion of this undertaking could not have been possible without the participation and assistance of so many people whose names may not all be enumerated. Their contributions are sincerely appreciated and gratefully acknowledged. However, I would like to express deep appreciation and indebtness particularly to the following:

1. Prof Madya Dr Mohd Ezane Aziz as the main supervisor of this dissertation
2. Dr Nur Hartini Mohd Taib as co-supervisor of this dissertation.
3. Professor Dr. Ibrahim Lutfi Shuaib, Prof Madya Dr Mohd Shafie Abdullah, Dr Juhara Haron, Dr Khairil Amir Sayuti, Dr Ahmad Tarmizi Musa, Dr Wan Aireene Wan Ahmed and Dr Ahmad Hadif Zaidin Samsudin, lecturers/radiologists all of whom directly or indirectly contributed their ideas and comments to the success of this study.
4. Puan Wan Nazyrah, radiographer incharge for MRI.
5. Puan Salmi Ab Aziz, research assistant for this dissertation.
6. Colleagues and all the staff in the Department of Radiology, HUSM.
7. RUI grant (1001/PPSP/812209) as the main sponsor of this study.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

DTI	Diffusion tensor imaging
MRI	Magnetic resonance imaging
FA	Fractional anisotropy
ADC	Apparent diffusion coefficient
SNR	Signal to noise ratio
ROI	Region of interest
SENSE	Sensitivity-encoding
SCM	Subscapularis muscle
SSM	Supraspinatus muscle
ISM	Infraspinatus muscle
TMM	Teres minor muscle

ABSTRAK

Latar belakang kajian: Imbasan pengimejan tensor difusi (DTI) merupakan satu kaedah standard untuk penilaian penyakit musculoskeletal. Tujuan kajian ini adalah untuk menentukan optimal nilai-b dan bilangan arah difusi untuk imbasan pengimejan tensor difusi (DTI) bagi otot rotator cuff dalam subjek yang sihat menggunakan sistem pengimejan resonans magnet (MRI) 3 Tesla.

Kaedah: Seramai 38 orang sukarelawan yang sihat telah dimasukkan di dalam kajian prospektif ini. Imbasan pengimejan tensor difusi (DTI) bagi otot rotator cuff (otot subscapularis, supraspinatus dan infraspinatus) telah dijalankan menggunakan urutan imbasan single-shot spin-echo echo-planar bagi 16 dan 32 bilangan arah difusi. Tiga nilai-b yang berlainan telah dipilih bagi setiap arah difusi : nilai-b 400 s/mm^2, 600 s/mm^2 dan $800s/mm^2$. Pengukuran nisbah isyarat-hingar (SNR) telah dikira bagi setiap otot untuk setiap nilai-b dan bilangan arah difusi yang berlainan dan purata SNR telah dikira. Analisis statistik telah dijalankan menggunakan ujian ANOVA sehala dan t berpasangan. Nilai min dan SD telah diperolehi bagi setiap parameter DTI dan tahap kepentingan telah ditentukan ($p < 0.05$).

Keputusan: Nilai SNR adalah paling tinggi untuk semua tiga otot menggunakan nilai-b $400s/mm^2$ (otot subscapularis, 40.255; supraspinatus, 38.203; and infraspinatus, 48.232), diikuti oleh nilai-b $600s/mm^2$ dan $800s/mm^2$. Penggunaan 32 bilangan arah difusi telah meningkatkan nilai SNR berbanding penggunaan 16 bilangan arah difusi ($P<0.05$).

Kesimpulan: Gabungan optimum bagi nilai-b dan bilangan arah difusi dalam imbasan DTI otot rotator cuff menggunakan MRI 3.0 Tesla masing-masing adalah 400s/mm^2 dan 32 arah difusi.

Kata kunci: Imbasan pengimejan tensor difusi (DTI), otot rotator cuff, nilai-b, bilangan arah difusi, nisbah isyarat-hingar (SNR)

ABSTRACT

Background: Diffusion tensor imaging is a standard method for evaluation of musculoskeletal pathology. The objective of this study is to determine the optimal b-value and number of diffusion direction for diffusion tensor imaging of rotator cuff muscles in healthy subjects on 3.0 Tesla MRI.

Methodology: 38 healthy volunteers were included in this prospective study. Diffusion tensor imaging (DTI) of the rotator cuff muscles (subscapularis, supraspinatus and infraspinatus muscles) was performed with single-shot spin-echo echo-planar imaging sequences in 16 and 32 diffusion directions. Three different b-values were selected for each diffusion direction: b-value 400 s/mm^2, 600s/mm^2 and 800s/mm^2. Signal to noise ratio (SNR) was measured in each muscle at different b-values and number of diffusion direction, and average SNR were calculated. Statistical analysis was performed with one-way ANOVA and paired t-test. Mean and standard deviation were obtained for each DTI parameters, and level of significance was determined ($p < 0.05$).

Results: The signal to noise ratio was highest for all three muscles at b-value 400s/mm^2 (subscapularis, 40.255; supraspinatus, 38.203; and infraspinatus, 48.232) followed by b-value 600s/mm^2 and 800s/mm^2. The use of 32 number of diffusion directions had shown to improve SNR value compared to 16 diffusion directions ($P<0.05$).

Conclusions: The optimal combination of b value and number of diffusion directions in DTI of rotator cuff muscles on 3.0 Tesla MRI is 400s/mm^2 and 32 directions respectively.

Keywords: diffusion tensor imaging (DTI), rotator cuff muscle, b value, number of diffusion direction, signal to noise ratio (SNR)

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Since the introduction of the magnetic resonance concept nearly half a century ago, magnetic resonance imaging (MRI) has rapidly evolved as a powerful imaging technique for evaluation of various tissues in-vivo. It uses strong magnetic field, radio frequency pulses, field gradients and a computer to generate detailed anatomical images in multiple planes without repositioning the patient. MRI also has superior soft tissue contrast compared to other imaging modalities such as computed tomography (CT) and ultrasound imaging. Advanced techniques such as diffusion, spectroscopy and perfusion allow precise tissue characterization for better understanding of the disease process.

Over many years, diffusion tensor imaging (DTI) has extensively been use for detection and characterization of brain tissue pathology at the microscopic level. Its use has been widened for investigation of musculoskeletal disease. DTI is able to provide some specific information of skeletal muscle and allows the detection of abnormalities at early stage where no morphological changes are detected on conventional MRI. The non-invasive character and the ability to distinguish between physiological changes and pathological process in skeletal muscle make this technique a standard method for in-vivo evaluation of musculoskeletal pathology.

The concept of diffusion weighted MRI was based on detection of the Brownian motion of water molecules. Diffusion is termed isotropic if the motion is equal in all directions. However, water diffusion in brain white matter and skeletal muscle exhibits directional dependence, also known as diffusion anisotropy because diffusion is restricted perpendicular to the long axis of the muscle fibers than along it. These anisotropic properties can be detected by DTI and may be used to define the direction of the fibers in a particular voxel.

A special feature of DTI that is not available in conventional MRI is the ability to perform muscle fiber tracking which is derived from post-processing of DTI data using special algorithm, therefore make it possible to acquire some diffusion values such as fractional anisotropy (FA) and apparent diffusion coefficient (ADC). The FA and ADC are frequently used to quantify the architectural changes in the tissues and the orientation of the muscle fibers. ADC and FA may vary depend on the magnetic field strength, voxel size, number of diffusion gradient, and signal-to-noise ratio.

DTI of skeletal muscles suffers from several technical challenges such as acquisition parameters, signal-to-noise ratio, image artifacts as well as fiber-tracking algorithms. There is still lack of studies regarding the optimal scan parameters for rotator cuff muscles. The muscle has low SNR compared with brain tissue. Thus, DTI acquisition parameters particularly b-value and number of diffusion directions must be further adapted to specific anatomical regions and type of MR equipment to improve SNR, reduce artifacts, optimizing spatial resolution and minimize the acquisition time.

B-value is an operator-selected parameter in DT-MRI and it reflects the strength, duration and timing of the gradients used to generate diffusion-weighted images. Higher b-value leads to stronger diffusion effect with concomitant increase in noise, thus result in decrease in signal-to-noise ratio (SNR) value of DTI data. The range of optimal b-value is not clearly defined and depends on MRI instruments (magnetic and gradient strength) and particular anatomical region being assessed. Another influencing factor on SNR is the number of diffusion gradient direction. The minimum 6 gradient directions are needed for calculation of diffusion tensor. As number of diffusion direction increases, more diffusion tensor images are used for the measurement of diffusion values, resulting in higher SNR and more precise diffusion values estimation.

From my literature review, many previous studies concentrate on DTI of lower limb muscles and spine. However, to the best of our knowledge, no such study conducted for the rotator cuff muscles, which is an important shoulder stabilizer. Therefore, it is of paramount importance to optimize both parameters in DT acquisition of rotator cuff muscles in order to achieve highest SNR for better image interpretation.

The internal arrangement and geometric properties of muscle fibers varies among muscles, which contributes to different diffusive properties of the muscle. DTI is essentially able to differentiate between functionally different muscles in the same region of the body. Thus, the optimized b-value and number of diffusion direction for DTI of the rotator cuff muscle obtained from this study allow for accurate determination of normative diffusion values, which would be beneficial for future reference in order to distinguish between normal and abnormal muscle fibers and helps in further treatment and prognosis of the injured muscle fiber.

Therefore, the aim of this study was to determine the optimal combination of b-values and number of diffusion directions for accurate DT imaging of rotator cuff muscles at 3.0 Tesla MRI. This is achieved by performing the SNR assessment of each muscle on each image acquisition.

1.2 OBJECTIVE

1.2.1 General objective:

To determine the optimal b-value and number of diffusion direction for diffusion tensor imaging of rotator cuff muscles of the shoulder in healthy subjects using 3.0 Tesla MRI.

1.2.2 Specific objectives:

1. To compare the signal to noise ratio (SNR) of rotator cuff muscle (subscapularis, supraspinatus and infraspinatus muscles) with different b-values and number of diffusion directions in healthy subjects.

2. To compare the signal to noise ratio (SNR) between different rotator cuff muscles (subscapularis, supraspinatus and infraspinatus muscles) in healthy subjects.

3. To compare the signal to noise ratio (SNR) of rotator cuff muscle (subscapularis, supraspinatus and infraspinatus muscles) with age, body mass index (BMI) and gender.

CHAPTER 2: LITERATURE REVIEW

DTI of Skeletal Muscle

Diffusion tensor imaging (DTI) is an advanced technique and one of the most powerful imaging tools for detection and characterization various pathology particularly central nervous system disorder. Based on the microscopic movements of water molecules at the cellular level, it allows the precise localization of white matter lesions' tract and helps in pre-surgical mapping of white matter tracts. DTI also provides advanced scientific understanding of many neurologic and psychiatric disorders. Besides cerebral imaging, application of DTI has been widened to study many other organs.

Recent advancement in DTI allows the implementation of this imaging technique in musculoskeletal disorder and its use has increased since past few decades. Diffusion imaging provides quantitative information on muscle geometry and internal architectural organization, which is the main mechanical determinant of muscle performance. Skeletal muscle is a highly ordered structure consist of elongated muscle fibers, appears as oblate polygon on cross section and bounded by a plasma membrane (Damon *et al*., 2011). Water diffusion in the skeletal muscle prefers the direction along the longitudinal axis of the fiber rather than perpendicular to it, thus creating anisotropic properties. DTI is a proven imaging technique to study the anisotropy and organization of structures in the skeletal muscle by providing a full three dimensional overview of the muscle of interest. Muscles of the lower limb have been in-depth studied (Sinha *et al.*, 2006, Kermarrec *et al*., 2010, Yanagisawa *et al*., 2009). The feasibility of in vivo DTI of human calf muscles also has been reported (Sinha *et al.*, 2006).

Rotator cuff muscles are an important group of muscles, connecting the upper arm (humerus) to the shoulder blade (scapula). The rotator cuff tendons provide stability to the shoulder. It consist of four muscles, namely supraspinatus muscle (SSM), infraspinatus muscle (ISM), teres minor muscle (TMM), and subscapularis muscle (SCM). Rotator cuff injuries are frequently sustained by athletes involved in repetitive overhead activities such as tennis player, baseball pitcher and weightlifter. As other muscle group, rotator cuff muscles also can involve in various pathologic conditions such as ischemia, inflammation and infection, besides traumatic induced. At present, many investigations are still relied on invasive muscle biopsy method, which has limitation in wide clinical application. Thus, non-invasive character of MRI offers a great advantage to study the physiology and morphology of the skeletal muscle. The early morphologic changes can be reliably detected by DTI. DTI is able to differentiate between functionally different muscles in the same region of the body based on their own diffusive tensor values (Galban *et al.*, 2004). Thus it is of paramount importance to determine normal diffusive values of the rotator cuff muscles for future reference to investigate abnormal muscle fibers. However, to the best of our knowledge, no such study conducted for the rotator cuff muscles.

Fiber Tractography

A 3-dimensional skeletal muscle architecture assessment using fiber tractography has opened a new window for non-invasive method to evaluate muscle fiber connectivity. Potential of DTI for tracking of muscle fibers both in animal and in-vivo has been shown in previous studies (Sinha *et al.*, 2006, Damon *et al.*, 2002). Budzik *et al*. (2007) has demonstrated in a DTI study of thigh muscles, that the muscle fibers in human subjects generated by tractographic analysis were correspond well with the anatomic region in dissected cadavers. All fibers were found to be well aligned within the muscle boundaries in the corresponding T2 weighted images. The fibers were traced until the insertion site at the aponeurosis, however the exact origin and insertion site of the muscle fiber could not be identified as the tendons are not visualized in the images. They also found that MR tractography is a reliable method to determine the shape and orientation of the muscle fibers in thigh muscles, however it does not represent the histological muscle fibers (Budzik *et al*., 2007). DTI has promising for the detection of early abnormalities in muscle. However, the clinical use of DTI to evaluate musculoskeletal disease is still under evaluation, thus necessitate much work to be done to establish the role of DTI (Budzik *et al.*, 2014).

Diffusive Properties

The ability of DTI to reconstruct the trajectories of skeletal muscle fibers allows for detection of several physiological values (diffusion values) which have a great association with the muscle physiology and pathology. Eigenvalues (λ^1, λ^2, and λ^3) are used to characterize the relative probability of motion of water molecule for the three main directions. Generally, the λ^1 value represents the water diffusion along the long axis of the muscle fibers, while the λ^2, and λ^3 value indicate the orthogonal water diffusion to the direction of λ^1 in the muscle fibers. The sum of the eigenvalues is called the trace, while their average is called the mean diffusivity or apparent diffusion coefficient (ADC). ADC reflects the extent to which the movement of water molecules is freely moved. Fractional anisotropy (FA) represents the degree of tissue anisotropy with value ranges from 0 (total isotropic) to 1(total anisotropic). Both ADC and FA are frequently used to assess the muscle shape and the orientation of the muscle fibers. The value from these two parameters can be obtained by positioning one or more regions of interest (ROIs) on the native diffusion tensor images or 3D tractography images. Kermarrec *et al*. (2010) reported that the intra-observer reproducibility was better in ROIs measurement on DTI images than on tractography (Kermarrec *et al.,* 2010).

Many studies have been conducted to determine the normal value of FA and ADC in thigh (Budzik *et al.*, 2007) and calf (Sinha *et al.*, 2006, Saupe *et al.*, 2009, Zaraiskaya *et al.,* 2006) muscles. The findings of these studies have shown that the different muscles in the same anatomical region have different FA and ADC values. In general, ADC values for calf muscles are higher compared to thigh muscles. Budzik *et al*. (2007) demonstrates the FA values (b-value of 400 s/mm2 with 32 directions) for thigh muscles ranges from 0.27 ± 0.08 (vastus medialis muscle) to 0.38 ± 0.11 (short head of biceps femoris) (Budzik *et al.*, 2007). Sinha *et al*. (2006) reported that the FA values (b-value of 600 s/mm2) for calf muscles ranges from 0.18 ± 0.04 (lateral soleus muscle) to 0.33 ± 0.03 (lateral gastrocnemius muscle) (Sinha et al., 2006).

The feasibility of pennation angle measurement of skeletal muscle in human using fiber tracking from DTI has been studied (Sinha *et al.*, 2006). The validity of pennation angle has been established in animal study by direct anatomical inspection of lateral gastrocnemius muscle (Damon *et al*., 2002).

Clinical Application of DTI of Muscles

As other tissues in human body, skeletal muscle fibers could not escape from the morphologic changes secondary to disease, lifestyle, environment and aging. Thus, the diffusion values for each muscle are also altered. ADC differences in skeletal muscles between genders, age groups and muscles had been studied (Yanagisawa *et al*., 2009). They studied the right ankle dorsiflexor muscles (tibialis anterior, extensor hallucis longus and extensor digitorum longus) of 166 healthy subjects and right erector spinae muscle of 86 healthy subjects. In this study, the ADC values of each muscle did not differ significantly between genders and age groups, except for the age group of 10-19 years which were significantly lower than those of the other groups. The erector spinae muscles showed significantly higher mean ADC values (b-value from 0 to 750 s/mm^2) than ankle dorsifexor muscles (1.51 $\pm$ 0.12 mm^2/s and 1.45 $\pm$ 0.05 mm^2/s, respectively).

The eigenvalues of the skeletal muscles are found to be sensitive to the age of subjects according to a study on human calf muscle (Galban *et al*., 2006). Linear regression of the principal eigenvalue (λ^1) of calf muscle with age was observed except for anterior tibialis muscle where the eigenvalues were smaller in the young group than in the older groups. In a study by Kermarrec *et al.* (2010) using 1.5 T demonstrated that the mean ADC and FA of the hamstrings and quadriceps femoris muscles were not significantly different with gender and age. The mean ADC and FA in women were 1.8 x 10^{-3} mm^2/s and 0.26, compared with men 1.79 x 10^{-3} mm^2/s and 0.26, respectively. They also noted that the quadriceps femoris and hamstrings muscles did have different ADC (1.91 x 10^{-3} mm^2/s vs. 1.64 x 10^{-3} mm^2/s) and FA (0.25 vs. 0.28) values. These findings may reflect the differences in hydration and muscular microarchitecture (Kermarrec *et al*., 2010).

MR tractography of skeletal muscle are also affected by gender (Okamoto *et al.*, 2010). In a study of human calf muscle, they found that the visualization of the muscle fiber tract in women is significantly better compared to men, particularly in soleus muscle. No significant difference of fiber tracts visualization by age was observed. The difference in diffusion properties of lower leg musculature between athletes and non-athletes had been studied (Okamoto *et al.*, 2012). There were statistically different of all three eigenvalues and ADC in all muscle of bilateral calves, in which the eigenvalues and ADC were lower in athletes than in non-athletes. However, FA showed no significant differences in any muscle. They postulated that in athletes, there is increased density of myofilaments in the intracellular space, and deformation of cell induced by enlargement of extracellular components.

Changes in diffusive parameters in pathologic conditions of the skeletal muscle also have been described (Zaraiskaya *et al.*, 2006). They evaluate the human skeletal muscle injuries using DTI and observed the significant differences in FA and ADC values in injured skeletal muscle compared to healthy subjects. In all patients, FA values were reduced compared to controls, to as low as 0.08 ± 0.02. Nevertheless the ADC values are consistently higher in injured skeletal muscle. The 2D projection maps demonstrate disorganized muscle fiber in injured calves while in healthy controls, a well ordered muscle fiber structure is observed. In an animal study using rabbit model, Zeng *et al*. (2006) had used DTI to distinguish the oedema, injury and rupture in skeletal muscle trauma. They found that there is significant difference between groups regarding FA and ADC values. In the tractographic analysis, they observed that muscle fiber tracking is slightly decreased in oedematous muscle and markedly decreased in injured muscle (Zeng *et al.*, 2006).

Factors Influencing SNR

In DT-MRI, the signal-to-noise ratio (SNR) is an important standard used to describe the performance of a MRI system, and is frequently used for image evaluation, measurement of contrast enhancement and quality assurance. The SNR measurement should be optimize in DT MRI sequences for precise investigation of muscle tissue. Various methods for SNR calculation have been described in detail (Dietrich *et al.*, 2007). The most commonly used technique is positioning of two separate regions of interest (ROIs) in a single image; one ROI in the tissue of interest to determine the signal intensity, and another ROI in the image background near to the anatomic region to measure the noise. The other alternative methods include the application of certain reconstruction filters, multichannel reconstruction or parallel imaging.

In general, the ideal SNR of DTI images should be more than 40 or at least 20 to prevent inaccurate or bias measurement of diffusive properties such as FA and ADC (Bastin *et al*., 1998, Mukherjee *et al.*, 2008). At smaller values of SNR (less than 20), the measurement of eigenvalues of diffusion tensor are prone to error, leading to overestimation of FA and underestimation of mean diffusivity. This results in problem to differentiate the isotropic regions with highly diffusion anisotropic tissues. A number of factors that contributes to improved SNR such as lower b-value, increase number of diffusion direction, use of higher magnetic field and increase voxel size (Sinha *et al*., 2006, Saupe *et al.*, 2009, Taib *et al.*, 2017).

The term "b-value" was first introduced in 1965. It is a factor that depends on the strength, duration and timing of the gradients used to generate diffusion-weighted images. It has been understood that the higher the b-value, the stronger the diffusion effects will be. However, the resultant increase in noise causes reduction of SNR value of DWI images (Sinha *et al.*, 2006, Saupe *et al.*, 2009). There is no specific b-value that is ideal for all type of DTI examination. It depends on the particular anatomical region being evaluated, magnetic field strength and types of MRI scanner. Optimal range of b-value for cerebral imaging has been well described in the literature (Taib *et al*., 2017, Toyoda *et al*., 2007). Previous studies on DTI of skeletal muscles (Galban *et al.*, 2004, Budzik *et al.,* 2007, Zaraiskaya *et al.*, 2006, Galban *et al.,* 2006, Zeng *et al.,* 2006) shows that the b-values used for DT MRI have ranged between 400 and 600 s/mm2 to obtain sufficient SNR for fiber tracking.

The optimal b-value for DTI and tractography of human calf muscles at 1.5 T also has been published (Saupe *et al.,* 2009). In the study, they also found that no significant different of FA with different b-values in anterior tibialis and lateral gastrocnemius muscles. However, a significant different was observed in fiber density index of the two muscles. The SNR was observed to reduce with increasing b-values. They found that the optimal b-value for DTI of calf muscles is 625s/mm^2. However, there is still lack of information about optimal ranges of b-values for rotator cuff muscles.

In DT-MRI, the signals are acquired by applying diffusion-sensitizing gradients along several non-collinear directions. The use of different number of diffusion direction has significant effect on certain diffusive tensor properties. A study by Ni *et al.* (2006) on human brain has found that the use of different number of diffusion directions in DTI sequence resulted in similar values of FA and mean diffusivity but different eigenvalues (Ni *et al.*, 2006). The minimum 6 gradient directions are needed for calculation of diffusion tensor. Larger number of diffusion directions will give increase SNR value due to more DTI images are used for the measurement of diffusion values. Therefore, determination of diffusive properties would be more accurate and precise. In a computer simulation study by Jones *et al*. (2004) it has been demonstrated that at least 20 unique sampling orientations are necessary for a robust estimation of anisotropy, whereas at least 30 unique sampling orientations are needed for a robust estimation of tensor-orientation (primary eigenvector), FA and mean diffusivity (Jones, 2004). Mukherjee *et al.* (2008) in their published article has recommended using 30 diffusion directions for clinical DTI even it takes longer acquisition time (Mukherjee *et al.*, 2008).

Technical consideration

Most of the diffusion imaging is performed by using spin-echo single-shot echo-planar imaging (SS-EPI) techniques. This ultrafast imaging method is able to produce 2D image from a single radiofrequency excitation pulse in a fraction of a second. Therefore, the motion artefact due to patient movements or even physiologic cardiorespiratory pumping during acquisition of DWI is significantly reduced. Another advantage of SS-EPI is relatively high SNR per unit of scanning time (Mukherjee *et al.*, 2008). This is particularly important in skeletal muscle imaging due to decrease SNR of muscle as a result of low T2 signal. Hence this technique offers a great benefit to scan the uncooperative patients or large body areas in a short time where other imaging modality fails to do so.

A common problem encountered when using SS-EPI method is the image distortion produced at higher field strength due to higher magnetic field inhomogeneity. A study for the brain showed that the SNR of the DW images is increased approximately two-fold from 1.5 T to 3.0 T, but echo planar image distortion is roughly doubled from 1.5 T to 3.0 T. However, this distortion is reduced by 50% when parallel imaging was used (Alexander *et al.*, 2006). Parallel imaging techniques such as sensitivity encoding (SENSE) and generalized auto-calibrating partially parallel acquisition (GRAPPA) can mitigate the image distortion and contrast blurring that occurs with SS-EPI method. The chosen acceleration factor used in parallel imaging must consider the substantial loss of SNR with higher acceleration. With the current MRI system, the acceleration factor of 2-3 is optimal (Mukherjee *et al*., 2008).

The diffusion properties of biological tissue are independent of magnetic field strength, but it does affect the SNR and artifacts of diffusion-weighted images. Stronger field strength enables greater diffusion-weighting in a shorter period of time. Saupe *et al*. (2008) compare the diffusion parameters in human calf muscle at 1.5 T and 3.0 T MRI. They found no significant different of eigenvalue, trace of the diffusion tensor, fractional anisotropy, relative anisotropy, volume ratio in 3 different calf muscle and b-value (b-value of 0, 300, 500 and 700) on 2 different field strengths. However, the SNR is significantly higher at 3.0 T compared with 1.5 T. Significant different of SNRs were observed at different b-value in different calf muscles. However, the number of sample in this study was very small (Saupe *et al.*, 2008).

DTI of skeletal muscles suffers from several technical challenges such as acquisition parameters, signal-to-noise, artifacts, underlying anatomical regions, as well as fiber-tracking algorithms. The muscle has low SNR compared with brain tissue due to low T2 signal in muscle, thus requiring use of lower b-value to increase SNR. Xu Longwei (2012) in his article review has provide a concise summary regarding several scan acquisition parameters of DTI of lower limb muscles using different MRI machine (Longwei, 2012). However, there is still lack of studies regarding the optimal scan parameters for rotator cuff muscles. Thus, DTI acquisition parameters particularly b-value and number of diffusion directions must be further optimized for this muscle group to improve SNR, reduce artifacts and minimize the acquisition time.

Therefore, the aim of this study was to determine the optimal combination of b-values and number of diffusion directions for accurate DT imaging of rotator cuff muscles at 3.0 Tesla MRI. This is achieved by performing the SNR assessment of each muscle on each image acquisition.

CHAPTER 3: STUDY PROTOCOL (METHODOLOGY)

3.1 Study Design

This was a cross sectional study which was conducted for a period of 24 months from June 2015 until June 2017. The study was held at the Radiology Department, Hospital Universiti Sains Malaysia (HUSM).

3.2 Sample Population

a) Reference population : All healthy adults

b) Source population : Healthy adult volunteers who are attended Hospital USM

3.3 Sampling Method

Convenience sampling method was used for this study. The volunteers were recruited until reached the required sample.

3.4 Sample Size

Sample size was calculated by using the G power software 3.1.9.2 that was downloaded from website http://www.gpower.hhu.de/en.html (see Appendix A). The sample size for objective 1, 2 and 3 are calculated using two means (paired). Standard deviation of difference is 2 and mean paired difference is 1, giving the effect size of 0.5. A prior power analysis indicated that 34 participants were sufficient to exhibit 0.8 power of the study. Considering a 20% of dropout, a total of 40 participants were needed for this study (power of study: 0.87). However we were able to get total of 38 volunteers, with the power of study 0.85.

3.5 Inclusion Criteria

1. Males and females adult healthy volunteers.
2. Age between 20 - 50 years old

3.6 Exclusion Criteria

1. Shoulder pain within the last 6 months.
2. Involved in strength or endurance training within the last 6 months.
3. History of shoulder trauma that necessitate medical attention.
4. History of shoulder surgery.
5. Findings of shoulder injury on MRI.
6. Contraindicated for MRI – ferromagnetic implant or devices

3.7 Methods

Advertisement of the study was made through email and flyers (see Appendix B) and the flyers were placed on the notice board in health campus Hospital Universiti Sains Malaysia. Interested volunteers aged 20-50 years old were carefully screened against the inclusion/ exclusion criteria. All participants were provided with detail explanation regarding the methodology of this study. Upon agreement, their informed consent were obtained (see Appendix C).

The participants were then given dates for MRI scanning which were held during weekend. The imaging was performed in Radiology Department, Hospital Universiti Sains Malaysia by experienced radiographer. Honorarium was given to each participant after the scan completed.

MRI of the rotator cuff muscles was performed using a MR scanner (Philips 3 Tesla Achieva MR scanner, Best, The Netherlands). The shoulder was placed in shoulder coil with arm placed at the side of the body in neutral position. A standard shoulder coil (SENSE_Shoulder 8) was used.

All subjects underwent the same imaging protocol. DT MRI was performed using single-shot spin-echo echo-planar imaging sequences in 16 and 32 diffusion directions (TR/TE 6750/72, field-of-view 200mm, matrix size 112x112, flip angle 90°, slice thickness/spacing 2.0/2.8mm, number of signal acquired 1, imaging time : 4 minutes and 10 seconds for 32 number of diffusion direction; 2 minutes and 10 seconds for 16 number of diffusion directions). Three different b-values were selected for each diffusion direction: b-value 400 s/mm^2, 600 s/mm^2 and 800s/mm^2. All image acquisitions had additional corresponding oblique sagittal T2 weighted image with a b-value of 0 s/mm^2.

The corresponding slice-matched T1 weighted images were obtained for better anatomic delineation to facilitate region-of-interest (ROI) analysis for each rotator cuff muscles. This MRI sequence was performed parallel to glenohumeral joint space. Twenty slices were acquired to cover the rotator cuff muscle from humeral tuberosities to the middle third of scapula. The sequence consist of :

1. oblique coronal 3D T1-weighted fast field echo (FFE) sequence (TR/TE 20/2, field-of-view 200mm, matrix size 320x320, flip angle 90°, slice thickness/spacing 1.4mm/0.7mm, number of signal acquired 2)
2. oblique sagittal T1-weighted turbo spin echo (TSE) sequence (TR/TE 869/20, field-of-view 150mm, matrix size 640x640, flip angle 90°, slice thickness/spacing 3.0/3.3mm, number of signal acquired 2).
3. axial T1-weighted turbo spin echo (TSE) sequence (TR/TE 700/20, field-of-view 160mm, matrix size 640x640, flip angle 90°, slice thickness/spacing 3.0/4.0mm, number of signal acquired 2).

The total acquisition time was 36 minutes for each volunteer. The DT images were then co-registered with baseline image (b=0) to correct the eddy-current-induced geometric distortion. Parallel imaging was performed using sensitivity-encoding (SENSE) algorithm with an acceleration factor of 2 to reduce total acquisition time and magnetic susceptibility artifact.

3.8 Image Analysis and Interpretation

Data analysis was carried out by researcher and verified by one musculoskeletal radiologist with 20 years of experience in musculoskeletal MRI. DTI image analyses were performed using Philips Extended MR WorkSpace 2.6.3.5, Best, The Netherlands software. A standardized circular ROI measured 300mm^2 was placed at the centre of three rotator cuff muscles at the scapula Y-view slice of the image date acquisition set (see Figure 1a-b). The three rotator muscles of interest were supraspinatus, infraspinatus and subscapularis muscle. The teres minor muscle was not included in this study to avoid inaccurate measurement due to small size of muscle and difficulty to place the ROI. The signal intensity and noise were measured on oblique sagittal plane with scapula Y-view (where the coracoid process and scapula spine met).

The ROIs were positioned within each muscle on the T2-weighted images (b=0) images and corresponding slice of the DT images in anatomically matched locations for each b-value and number of diffusion direction acquisition (b-values of 400, 600 and 800s/mm^2, number of diffusion direction 16 and 32) in each volunteers (see Figure 1a-b). The signal measurement was obtained three times in each muscle for each b-value and number of diffusion direction acquisition. The average signal intensity was then calculated for each muscle at each b-value and number of diffusion direction.

Noise was defined as the standard deviation of signal intensity in air outside the anatomic structures. For determination of noise, standardized ROI measuring 300mm^2 were placed in three reproducible locations in air outside the anatomic extremity, close to the image edge (Dietrich et al., 2007, Saupe et al., 2009) (see Figure 2a-b). The obtained noise value was averaged for each b-value and number of diffusion direction. Signal-to-noise ratio (SNR) was calculated in each muscles for each b-values and number of diffusion directions and mean SNR was acquired. SNR was calculated using the following equation (Saupe et al., 2009, Dietrich et al., 2007) :

$$\mathrm{SNR} = S_{\mathrm{m}}/SD_{\mathrm{n}}$$

S_{m}: signal intensity of muscle

SD_{n}: is the SD of signal in air equal to noise

3.9 Data Collection

Subject's information i.e. age, gender, body mass index (BMI), acquisition parameter (b-value, number of diffusion direction), ROI in mm^2 and SNR were recorded in the data collection sheet (see Appendix F) and labeled with serial number in order to maintain privacy and confidentiality of subject.

3.10 Confidentiality and Privacy

The participants were identified by a serial number. No identifiable data were shared publicly. The information were used for research purpose only and encrypted in a password protected medium. The data were retained by the researchers for knowledge purposes only. Neither the name nor any identifying information was used in any publication or presentation resulting from this study.

3.11 Statistical Analysis

All quantitative data were analyzed using Microsoft® Office Excel and Statistical Product and Service Solutions (SPSS) for Windows, SPSS Inc.© (version 24, SPSS Inc., Chicago, IL,USA). All data were expressed as mean (standard deviation) [mean (SD)]. Distribution of the data was examined prior to statistical analysis using different methods which include histogram with normal curve, homogeneity and linearity of the data. All normally distributed data was analyzed using parametric test which is paired-sample t-tests for different number of diffusion directions (16 and 32) and one way analysis of variance (ANOVA) test for different b-values (400, 600 and