APPARENT COEFFICIENT DIFFUSSION (ADC) AND FRACTIONAL ANISOTROPY (FA) VALUES OF THE ROTATOR CUFF MUSCLES USING DTI AT 3-TESLA MRI IN THE HEALTHY POPULATION

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR MASTER OF MEDICINE (RADIOLOGY)

UNIVERSITI SAINS MALAYSIA

2018

ACKNOWLEDGEMENT

The success of this undertaking could not have been completed without the participation and assistance of so many people whose names may not all be enumerated. Their input, guidance and assistance are sincerely appreciated and gratefully acknowledged. I would also like to express my deepest gratitude to the Human Research Ethic Committee of Universiti Sains Malaysia for approving my study and providing it with a research grant (JEPeM Code: USM/JEPeM/15120584) which complies with the Declaration of Helsinki.

I would like to extend my appreciation and gratitude particularly to the following:

1. Prof Madya Dr Mohd Ezane Aziz as supervisor and Prof Dr Hj Ibrahim Lutfi Shuib as well as Dr Nur Hartini Mohd Taib as co-supervisor of this dissertation.
2. Prof Madya Dr Mohd Shafie Abdullah, Dr Juhara Haron, Dr Khairil Amir, Dr Ahmad Tarmizi, Dr Wan Aireene and Dr Ahmad Hadif, lecturers/radiologists all of whom directly or indirectly contributed their ideas and comments to the success of this study.
3. Dr Esther Rishma Sundram as my statistical analyses guidance counsellor.
4. Puan Wan Nazyrah, radiographer in charge for MRI.
5. Puan Amysalmi, research assistant.
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 ACRONYMS

MRI Magnetic resonance imaging

DTI Diffusion tensor imaging

FA Fractional anisotropy

ADC Apparent diffusion coefficient

SNR Signal to noise ratio

ROI Region of interest

SENSE Sensitivity-encoding

SS Supraspinatus muscle

IS Infraspinatus muscle

SC Subscapularis muscle

ABSTRAK

Objektif: Untuk mengimbaskan nilai-nilai ADC dan FA bagi otot-otot 'rotator cuff' dalam subjek populasi yang sihat menggunakan sistem pengimejan resonans magnet (MRI) 3.0Tesla.

Kaedah: Beberapa nilai parameter boleh diperolehi daripada DTI data. Nilai-Eigen ((λ^1, λ^2, dan λ^3) digunakan untuk mencirikan pengerakan zarah air dalam tiga arah umum. Nilai-nilai lain yang boleh diperolehi adalah seperti purata diffusi, ADC dan FA. Seramai 38 orang sukarelawan yang sihat telah dimasukkan di dalam kajian prospektif bagi menentukan kadar mean ADC dan FA dalam bahu. 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 sebanyak 32 bilangan arah difusi, dengan nilai-b 400 s/mm^2 (dipilih sebagai kaedah paling optima untuk kajian ini). Jesteru itu nilai 'apparent coefficient diffusion' (ADC) dan nilai 'fractional ansotropy' (FA) diperolehi dari imej otot-otot dalam paparan 'sagittal-oblique' bahu. Data dikira mengunakan Philips Extended MR WorkSpace 2.6.3.5. Analisis statistik telah dijalankan menggunakan ujian ANOVA sehala melalui SPSS ver 24. Nilai min dan SD telah diperolehi bagi setiap parameter DTI, dan tahap kepentingan telah ditentukan ($P < 0.05$).

Keputusan: Purata ADC otot supraspinatus ialah 1.63 (±0.12), infraspinatus 1.61 (±0.09) dan subscapularis 1.74 (±0.15). Purata FA otot supraspinatus ialah 0.45 (±0.05), infraspinatus 0.41 (±0.36), subscapularis 0.47 (±0.55). Perbezaan purata ADC antara otot supraspinatus dan subskapularis serta otot subscapularis dan infraspinatus adalah berbeza dengan berdasarkan ujian Post-hoc Scheffe ANOVA One-Way. Maksud ADC antara otot supraspinatus dan infraspinatus bermakna perbezaannya sama. Perbezaan FA yang sama antara semua 3 pasang otot sangat berbeza. Pekali Intraclass untuk FA dan ADC antara 2 penilai menghasilkan keputusan berkisar baik dengan keputusan cemerlang.

Kesimpulan: Kajian ini berjaya menunjukkan nilai-nilai FA dan ADC dalam calon-calan yang ada dalam kategori sihat. Kajian juga berjaya menujukan dua-dua kaedah penilaian ini boleh dipercayai.

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

Objective: To determine the value of apparent diffusion coefficient (ADC), and fractional anisotropy (FA) of the rotator cuff muscles using a 3-Tesla MRI in healthy subjects and comparing its differences.

Materials and Methods: Thirty-eight healthy volunteers were included in this prospective study. Diffusion tensor magnetic resonance imaging (DT MRI) of the rotator cuff muscles were performed. Single-shot spin-echo echo-planar imaging sequences in 32 diffusion directions with a b-value of 400 s/mm^2, was selected as most optimal for this study. Subsequently ADC and FA values of the rotator cuff muscles were acquired from the sagittal oblique view of the shoulder by manually drawing an elliptical range of interest of the visualised muscles. Statistical analysis carried out using One-Way ANOVA post-hoc Scheffe test. Measurement was taken by two ratters to reduce bias.

Results: Mean ADC with standard deviation of the supraspinatus was 1.63 (±0.12), infraspinatus was 1.61 (±0.09) and subscapularis muscle was 1.74 (±0.15). Mean FA with standard deviation of the supraspinatus was 0.45 (±0.05), infraspinatus was 0.41 (±0.36), subscapularis muscle was 0.47 (±0.55). Mean difference of ADC between supraspinatus and subscapularis muscles as well as subscapularis and infraspinatus muscles were significantly different. The mean ADC between the Supraspinatus and infraspinatus muscles mean difference were similar. Mean difference of FA between all 3 pairs of muscles were significantly different. Intraclass Coefficient for FA and ADC between 2 Raters ranges from good to excellent results.

Conclusion: This study has been able to obtain normal range of values of FA and ADC. Among healthy subjects using DTI on a 3-Tesla MRI. These two means are reliable.

Keywords: *diffusion tensor imaging (DTI), rotator cuff muscle, fractional anisotropy, apparent diffusion coefficient*

CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) are widely used in the evaluation of the central nervous system. Apparent diffusion coefficient (ADC) is a measure of diffusion of water molecules within tissue using a diffusion weighted MR imaging (DW-MRI). DWI exploits the random motion of water. The impedance of water molecule is determined by tissue cellularity and presence of intact membranes. DWI has been used for the investigation of cerebral infarctions, tumor behavior in the brain and bone tumors. Recently it has been used to investigate other organs, such as muscle, prostate, breast, liver and pancreas.

Fractional anisotropy (FA), is a common measurement used in DTI study, and it quantifies the anisotropic direction of water molecules. FA is a measure of the degree of diffusion anisotropy, and it is calculated from the diffusivity constants λ^1, λ^2, and λ^3. It is a scalar value between zero and one that describes the degree of anisotropy of a diffusion process. A value of zero means that diffusion is isotropic, i.e. it is unrestricted (or equally restricted) in all directions.

DWI uses the random motion of water molecules and by implicating different b-values onto the DWI image, the assessment of ADC values can be evaluated from the MRI workstation. ADC values are calculated by software from the workstation and then values are displayed as parametric map which differentiates the degree of diffusion of water molecules in different tissues. ADC is defined in units of mm^2/s. The extent of tissue cellularity and the presence of intact cell membrane help determine the impedance of water molecule diffusion. This impedance of water molecules diffusion can be quantitatively assessed using the ADC value.

The rotator cuff muscles work in synchrony to provide movement of the shoulder as a whole, they consist of the supraspinatus muscle, infraspinatus muscle, subscapularis muscle and the teres minor muscle. Rotator cuff injuries are a common obstacle faced by those in the field of orthopedics and sports medicine. These injuries do not only include people who are active in sports but also those with occupations that involves repeated overhead movements such as painters, carpenters for example. Mainly treatment decisions should be based to eliminate the dysfunction by correcting the causes, to which clinicians historically have depended on the usage of corticosteroid injections. In a full thickness tear, the treatment option would more likely lean towards a surgical repair. However, the efficacy of such methods, can only be evaluated by clinically accessing the return or partial return of function to the group or specific muscle of the rotator cuff muscle.

DTI has shown to be a feasible for a non-invasive evaluation of the micro-architecture within most skeletal muscles. Several studies have been conducted to determine the ADC and FA of the calf and paraspinal muscles in the healthy population. These studies managed to determine the normal mean range of the healthy tissues, and there also were studies comparing specific muscles with injuries to that of normal ones. There is a study to compare the changes of DTI parameters difference in the shoulder for subjects undergoing body building training. However, to date no published articles has described the FA and ADC values within the normal population.

DTI parameters are dependent on several acquisition parameters such as echo time, b-value and diffusion mixing time. DTI values and parameters are also varying with age, body mass index and gender and other factors such as exercise, rest and even temperature. Most of a muscle fiber volume is occupied by the contractile protein filaments (myofibrils) and the water internal to and around them. These myofibrils are arranged parallel to each other. Using DTI, an advanced MRI imaging method fiber tracking of muscle fibers can be obtained. Hence the ability to quantify the diffusion of the muscle fibers is made possible, revealing diffusion values such as FA and ADC. Fiber tracking is able to assist in both localization and quantitative assessment of the muscle pathways. The diffusion within each voxel is used in fiber tracking to follow a tract in three dimensions (from voxel to voxel). Prior anatomical knowledge is important, especially so within the rotator cuff muscles, as the constraints of the muscle being angulated, a user-defined region of interest (ROI) is needed to accurately represent the different muscles which are of interest.

By determining the ADC and FA values in the healthy population, one is able to set a comparative standard when pathological values are measured. By studying these values for the muscles of the rotator cuff in the normal population, one is able to set a range of normal parametric values of FA and ADC for standardization. This will greatly aid in the post rehabilitation and post-operative cases involving patients who has sustained an injury to reevaluate the muscles FA and ADC values as a benchmark to recovery prognosis.

1.2 General objective:

To determine the value of apparent diffusion coefficient (ADC), and fractional anisotropy (FA) of the rotator cuff muscles using a 3-Tesla MRI in healthy subjects and comparing its differences.

1.3 Specific objectives:

1. To compare the ADC values in diffusion tensor (DT) images of rotator cuff muscle (subscapularis, supraspinatus and infraspinatus muscles) in healthy subjects.

2. To compare the FA of DT images of rotator cuff muscle (subscapularis, supraspinatus and infraspinatus muscles) in healthy subjects.

3. To determine inter-observer calculation value of ADC and FA between different rotator cuff muscles (subscapularis, supraspinatus and infraspinatus muscles).

CHAPTER 2: LITERATURE REVIEW

2.1 LITERATURE REVIEW

With the emergence and gaining popularity of diffusion tensor magnetic resonance imaging (DT MRI) as a formidable technique to study nerve tracts and muscles fibers at the molecular level, water molecule directional flow can be mapped into a three-dimensional plane. The concept of diffusion weighted MRI was based on detection of the Brownian motion of water molecules (Le Bihan and Iima, 2015). Water diffusion in anisotropic tissues (brain white matter and skeletal muscles) exhibits directional dependence, also known as diffusion anisotropy because diffusion is restricted perpendicular to the long axis of the muscle fibers than along it (Damon *et al.*, 2011).

Post processing of raw data obtained in the diffusion tensor imaging (DTI) sequence by means of a special algorithm making the ability to obtain diffusion values such as fractional anisotropy (FA) and apparent diffusion coefficient (ADC) making muscle fiber tracking possible. 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 (Alexander *et al.*, 2006).

Based on the diffusive tensor properties, DTI sequence has been widely applied to detect various neurological disease which has been well described in the literature. Its use in the investigation of skeletal muscle pathology has been increased since past few decades. Previous studies have shown a potential of DTI to be used for tracking of muscle fibers (Damon *et al.*, 2002; Shantanu *et al.*, 2006). Diffusion values of skeletal muscle fibers obtained from DTI technique enable early detection of pathological process in the muscle where the morphological changes are not detected by conventional MRI. However, the clinical use of DTI to evaluate musculoskeletal disease is still under assessment, thus necessitate much work to be done to establish the role of DTI (Budzik *et al.*, 2014). Despite that, the noninvasive feature and the ability to depict physiological changes (exercise-induced) and pathological process (inflammation, ischemia, trauma) will make this unique technique as a standard method for evaluation of musculoskeletal disease.

The signal to noise ratio (SNR) measurement should be optimize in diffusion tensor (DT) MRI sequences for investigation of muscle tissue. Methods for SNR calculation has been described in detail by (Dietrich *et al.*, 2007), which includes the conventional and alternative method by 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 (Mukherjee *et al.*, 2008b). Low SNR value will lead to overestimation of FA and underestimation of eigenvalues and mean diffusivity. A number of factors that contributes to improved SNR such as lower b value, increase number of diffusion direction, use of higher magnetic field (Saupe *et al.*, 2009; Sinha *et al.*, 2006) and increase voxel size (Mohd 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. The higher the b-value, the stronger the diffusion effects which resulted in reduction of SNR value of diffusion-weighted imaging (DWI) (Saupe *et al.*, 2009; Sinha *et al.*, 2006). 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. Previous studies on DTI of skeletal muscles (Galban *et al.*, 2004; Tatiana *et al.*, 2006) shows that the b values used for DT MRI have ranged between 400 and 600 s/mm^2. The optimal b-value for human skeletal muscles in the calf at 1.5 T also has been published (Saupe *et al.*, 2009). However, there is still a lack of information about optimal ranges of b values for rotator cuff muscles. The probable reasons could be due to the muscle position and curved anatomical position from its origin to insertion point.

Clinically ADC has been described to quantify a voxel-based morphometry that shows a difference of value when comparing pathological brain with Alzheimer's to a normal one (Takahashi *et al.*, 2017). In the early 1930's chemists recognized that the measured diffusion rates of dyes and other small molecules were highly dependent both on the nature of the media studied and on experimental conditions. Recognizing these limitations, they began to use the term ADC to refer to estimated diffusion rates obtained from their studies. In the modern MR literature, the term ADC is used in at least two different contexts. In the most general sense ADC is synonymous with measured/observed diffusion rates or tensor components, reflecting the methodological uncertainties described above. In other contexts, ADC is used to refer to the mean diffusion in a voxel, sometimes taken as the sum or average value of the tensor's diagonal elements. The commonly viewed ADC map reflects this latter definition (Le Bihan and Iima, 2015).

In DT-MRI, the signals are acquired by applying diffusion-sensitizing gradients along at least six 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. Monte Carlo computer simulation study has demonstrates 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.*, 2008b) in their published article has recommended using 30 diffusion directions for clinical DTI even it takes longer acquisition time.

Previous authors had described DT MRI imaging involving the muscles of the thigh of five subjects and assess the values of the tractography images, namely the ADC and FA values (Budzik *et al.*, 2007). The mean values obtained from the cited study were listed into a table as follows.;

Thigh Muscles	**ADC $10^{-3}mm^2/s$**	**FA**
Sartorius	0.91 ± 0.26	0.35 ± 0.09
Gracilis	0.76 ± 0.23	0.35 ± 0.11
Rectus femoris	0.92 ± 0.20	0.29 ± 0.08
Vastus intermedius	0.96 ± 0.24	0.28 ± 0.08
Vastus medialis	0.87 ± 0.21	0.27 ± 0.08
Vastus lateralis	0.92 ± 0.21	0.27 ± 0.09
Adductus	0.96 ± 0.26	0.28 ± 0.09
Biceps femoris short head	0.90 ± 0.29	0.38 ± 0.11
Biceps femoris long head	0.89 ± 0.31	0.29 ± 0.10
Semitendinous	0.88 ± 0.31	0.30 ± 0.09
Semimembranous	0.85 ± 0.30	0.34 ± 0.10

These values were obtained using a 1.5T MRI, however the aim of the study was to prove the feasibility of providing in vivo 3D architecture of the human thigh and determining muscular microstructural parameters (ADC and FA) (Budzik *et al.*, 2007).

Within muscle fibers diffusion of water can be measured when a weighted image is sequenced with more than six diffusion directions and thus, producing fiber tractography via means of diffusion tensor imaging (Le Bihan and Iima, 2015; Saupe *et al.*, 2009). By that the ADC and FA values can then be analysed and quantified (Galban *et al.*, 2004). It has also been noted in the study conducted by (Okamoto *et al.*, 2010) that there were differences in perfusion of the muscles between rest and contraction.

DTI performed using echo-planar imaging (EPI) method produced more distorted images at higher field strength due to higher magnetic field inhomogeneity. A study of the brain showed that the SNR of the DW images are increased approximately two-folds 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).

Literature (Saupe *et al.*, 2009) compares 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 signal to noise ratios were observed at different b-value in different calf muscles. A major fallback to those results were that the number of samples in that study was very small. The idea is to provide quantitative parameters that can be used as a mean to evaluate and compare normal muscle fibers. Within muscle fibers diffusion of water can be measured when a weighted image is sequenced with more than six diffusion directions and thus, producing fiber tractography via means of diffusion tensor imaging (DTI) (Le Bihan and Iima, 2015; Saupe *et al.*, 2009). Therefore ADC and FA values can then be analysed and quantified (Galban *et al.*, 2004). It has also been noted in the study conducted by (Okamoto *et al.*, 2010) that there were differences in perfusion of the muscles between rest and contraction.

Rotator cuff muscles is an important group of muscles that act as shoulder stabilizer. It consists of four muscles, namely supraspinatus muscle, infraspinatus muscle, teres minor muscle, and subscapularis muscle. Rotator cuff injuries are frequently sustained by athletes involved in repetitive overhead activities such as tennis player, baseball pitcher and weightlifter, other professions such as painters and carpenters also are susceptible to injuries. Subtle injury to the rotator cuff muscles can be detected using DTI based on changes of diffusive properties which is decrease FA and increase ADC value (Tatiana *et al.*, 2006).

Different muscles in human body has its own diffusive properties and DTI is essentially able to differentiate between functionally different muscles in the same region of the body (Galban *et al.*, 2004). Thus, normative diffusion values in different groups of muscle should be obtained 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. DTI of the lower limb muscle such as calf muscles, thigh and erector spinae spine had been in-depth studied (Kermarrec *et al.*, 2010; Shantanu *et al.*, 2006; Yanagisawa *et al.*, 2009). However, to the best of our knowledge, no such study has been conducted for the rotator cuff muscles.

The objective of having to normally quantify the ADC and FA is to one day compare the normal with pathological muscles of the rotator cuff. As described by (Tatiana *et al.*, 2006) in her comparison between healthy and injured calf muscles results in a clear difference in the FA and ADC values of those muscles.

There were several technical challenges of DTI for the muscles, such as acquisition parameters, signal-to-noise, artifacts, underlying anatomical regions, as well as fiber-tracking algorithms. The muscles had low SNR compared with brain tissue due to low T2 signal in muscle, thus requiring use of lower b value to increase SNR. Literature (Longwei, 2012) has provided a concise summary regarding several scan parameters of DTI of lower limb skeletal muscles using z different MRI machine. 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 (Saupe *et al.*, 2009).

Differences between the dominant and non-dominant hand muscle strengths were proved to be significant in all age groups (Sebastjan *et al.*, 2017). One of the different parameters comparing age-related changes in dominant and non-dominant hands were use-dependent plasticity amongst subjects.

Determining the optimal combination of b values and number of diffusion directions for accurate diffusion tensor imaging of rotator cuff muscles on 3.0 Tesla MRI. This is achieved by performing the SNR assessment of each muscle on each image acquisition. After which an elliptical measurement is made over the desired muscles groups in the sagittal oblique view to generate ADC and FA values based on the best SNR values obtained. These values are then cross analysed to further demonstrate their significance or difference.

CHAPTER 3: METHODOLOGY

3.1 Study design:

This is a cross sectional study that was conducted in Radiology Department, Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan. The ethical approval was obtained from Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM Code: USM/JEPeM/15120584) which complies with the Declaration of Helsinki (Appendix 6.1).

3.2 Sample Population:

All healthy adults in the Kubang Kerian region and HUSM.

3.3 Sampling technique:

Convenience sampling

Advertisement of the study was made through email and flyers (see appendix 6.4) 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. Subjects who were in a healthy physical state with no prior injuries or surgery to their non-dominant hand were of the main criteria, as the difference in age related changes and use-dependency may vary in dominant hands (see study protocol: inclusion and exclusion criteria). All participants were provided with detail explanation regarding the methodology of this study. Upon agreement, their informed consent was obtained. All consents were logged and filed.

3.4 Inclusion Criteria:

1. Healthy male and female adult volunteers.
2. Age between 20 - 50 years old
3. Non-dominant hand

3.5 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. Dominant hand.
6. Findings of shoulder injury on MRI.
7. Contraindicated for MRI – ferromagnetic implant or devices

3.6 Sample Size Calculation:

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 6.2). The sample size for objective 1 and 2 were calculated for two means (paired). Standard deviation of difference is 2 and mean paired difference is 1, giving the effect size of 0.5. Calculation indicated that 27 participants were sufficient to exhibit 0.8 power of the study. Considering a 20% of dropout, a total of 27 participants were needed for this study (power of study: 0.81). A total of 38 volunteers were enrolled into this study.

$$\rho = \frac{\sigma_B^2}{\sigma_W^2 + \sigma_B^2}$$

In the equation, σ_B represents the standard deviation between the raters and σ_W indicates the standard deviation within the raters. To show the magnitude of agreement between two raters for this study, many research designs require assesments of ICC (Hallgren, 2012).

3.7 Research Tools:

1. MRI machine – Philips 3 Tesla Achieva MR scanner, Best, The Netherlands. A standard shoulder coil was used.

2. MR sequences:

- Sagittal oblique diffusion weighted image for measurement
- Pulse sequence : single-shot spin-echo, echo-planar imaging
- Coil: SENSE-Shoulder 8
- Field of view (FOV): 200
- Matrix size: 112 x 112
- TR/TE (ms): 6750/72
- Flip angle: 90°
- Slice thickness (mm): 2.0
- Slice gap (mm): 2.8
- Number of signal acquired: 1
- Imaging time: 4 minutes and 10 seconds (for 32 diffusion directions)
  2 minutes and 5 seconds (for 16 diffusion directions)

3. Workstation – Philips Extended MR Workspace 2.6.3.5, Best, The Netherlands.

3.8 Operational Definition

Apparent diffusion coefficient (ADC): is a measure of the magnitude of diffusion of water molecules within tissue. It is commonly clinically calculated using MRI with diffusion weighted imaging (DWI). DWI exploits the random motion of water molecules. The extent of tissue cellularity and the presence of intact cell membrane help determine the impedance of water molecule diffusion. This impedance of water molecules diffusion can be quantitatively assessed using the apparent diffusion coefficient (ADC) value. ADC values are calculated automatically by the software and then displayed as a parametric map that reflects the degree of diffusion of water molecules.

Fractional Anisotropy (FA): is a measure of the degree of diffusion anisotropy, and it is calculated from the diffusivity constants λ^1, λ^2, and λ^3 .It is a scalar value between zero and one that describes the degree of anisotropy of a diffusion process. A value of zero means that diffusion is isotropic, i.e. it is unrestricted (or equally restricted) in all directions.

Rotator cuff muscles : a group of tendons and muscles in the shoulder, connecting the upper arm (humerus) to the shoulder blade (scapula). The rotator cuff tendons provide stability to the shoulder; the muscles allow the shoulder to rotate. The muscles in the rotator cuff include subscapularis, supraspinatus, infraspinatus and teres minor muscles. However the teres minor muscle was not included in this study

B value : B value is a factor that characterize the degree of diffusion weighted that reflects the strength, duration and timing of the gradients used to generate diffusion-weighted images. The higher the *b*-value, the stronger the diffusion effects.

Diffusion Weighted Images (DWI): occurs when random Brownian motion of water molecules is imaged onto a voxel of tissue. Often clinical use is to differentiate a higher diffusion in pathological states in comparison to normal diffusion in healthy tissue.

Number of diffusion direction : It is the number of direction of diffusion-encoding gradient that is selected by the user. At least 6 number of diffusion direction is needed to calculate the diffusion tensor values.

Diffusion Tensor Magnetic Resonance imaging (DT MRI) : Diffusion refers to the water movement as describe as Brownian movement of particles. When an image is not weighted then it is referred to have a b value of 0. When the b-value is greater than 0, the image is then described as weighted. Now when diffusion is applied onto at least non-co-linear directions, for each pixel that the image is produced, it is possible to calculate the diffusion tensor that describes the diffusion anisotropy of the matter (i.e. brain, muscle). The theory then expands into the movement of water within the myelin sheath for the brain or myofibrils in the skeletal muscles producing a fiber tract (Lemaire *et al.*, 2011).

Signal to noise ratio (SNR) : it is the standard parameter to describe the performance of an MRI system and frequently used for image evaluation, measurement of contrast enhancement and quality assurance. It is measured by dividing the signal intensity of the tissue with the background noise.

Range of interest (ROI) : in magnetic resonance range of interest describes area of the image that is of analytical value to be circled or elliptically outlined to perform data analysis.

3.9 Image Acquisition of the subject:

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 (Neutral position being an outstretched arm with anatomical supination making the radius and thumb the most lateral structures). A standard shoulder coil (SENSE_Shoulder 8) was used. 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.

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. During the study image acquisition 2 diffusion directions (16 and 32) with each weighted with 3 different b-values (400, 600, 800s/mm^2) and it was analysed which of it would produce the best signal to noise ration prior to proceeding analyzing diffusion tensor values of the muscles. Material for selection of 32 directions with a b value of 400s/mm^2 demonstrating the best signal to noise ratio is under the consideration of publication. All image acquisitions had additional corresponding oblique sagittal T1 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 (Saupe *et al.*, 2009). 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 sequences consisted 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.

3.10 Image Analysis

All raw data from the MR scanner (Philips 3 Tesla Achieva MR scanner, Best, The Netherlands) is transferred into the designated workstation (Workstation – Philips Extended MR Workspace 2.6.3.5, Best, The Netherlands). This was to ensure a controlled environment which the images are loaded onto, processed and analysed. A standardized circular range of interest (ROI) was 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 with same ROI area placed onto the slice with no data to obtain a signal to noise ratio. The signal to noise ratio was then measured and analysed to obtain which b-value and diffusion directions produced the best results. The result, raw image from 32 diffusion directions with a b-value of 400 s/mm^2 were selected and loaded onto the workstation screen (data pending publication).

Data analysis was carried out by researcher and one musculoskeletal radiologist with 20 years of experience in musculoskeletal MRI. DTI image analysis were performed using Philips Extended MR WorkSpace 2.6.3.5, Best, The Netherlands software. 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 range of interest ROI. On the tools option of the workstation Fibertrak option is selected and the raw data from the diffusion tensor images were processed.

Diffusion tensor images were slice-matched with the corresponding T1 weighted images for better anatomic delineation to facilitate region-of-interest (ROI) analysis for each rotator cuff muscles (Saupe *et al.*, 2009)). The images were analysed on the Philips Extended MR Workspace 2.6.3.5, Best, The Netherlands. Manual outlining of each selected muscle group was selected after full view of the scapula-Y on its sagittal oblique slice.

On the sagittal oblique scapula Y-view the ROI's were positioned within each muscle group on the T1-weighted images and the corresponding slices of the diffusion tensor images (DTI) in anatomically matched locations in each of the volunteers (Figures 1, 2 and 3). Elliptical drawing by each observer was made and the ADC and FA values were produced. The generated measurement was obtained in each muscle group. The values were then divided and recorded into each muscle group of the rotator cuff (supraspinatus, infraspinatus and subscapularis), with two data sheets labelled as Rater 1 and Rater 2 (Appendix 6.2).

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). The data were expressed as means ± S.Ds. Parametric test was used for analysis. All statistical tests were considered significant when the p value was < 0.05.
One-way ANOVA with post-hoc Scheffe test was performed on the acquired data.

Interclass correlation coefficient (ICC) is a widely used reliability index in test-retest, intrarater, and interrater reliability analyses. This article introduces the basic concept of interclass coefficient ICC for the purpose of analysis towards the content of reliability analysis (https://www.ncbi.nlm.nih.gov/pubmed/27330520) (Koo and Li, 2016). This study incorporates data analysed by 2 raters, making it an interrater reliability analysis to reduce bias of the results. Based on the 95% confident interval of the ICC estimate, values less than 0.50, between 0.5 and 0.75, between 0.75 and 0.90, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively. Methods employed to justify that measurement tools which were used to quantify the variable provides a stable or consistent response in other words, reliability. The term reliability then refers to results that were obtained in a measurement and to what degree it can be replicated (Bolarinwa, 2015). In order to provide a good reliability to the study a clear methodology was described and more importantly an inter-rater reliability method was employed. ICC method described by (Koo and Li, 2016) provided the practical guideline required to strengthen the research reliability overall.