QUANTITATIVE MRI FIBRE TRACTOGRAPHY IN LEUKOARAIOSIS: ANALYSIS OF LESIONAL AND NON-LESIONAL CEREBRAL WHITE MATTER

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DISCLAIMER

I declare that this dissertation records the results of the study performed by me and that it is of my own composition.

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(MOHD AZAAD BIN A HAMID)

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

MRI	Magnetic Resonance Imaging
DTI	Diffusion Tensor Imaging
PACS	Picture archieve communication system
ROI	Region of interest
ADC	Apparent diffusion coefficient
FA	Fractional anisotrophy
FDI	Fiber Density Index

TRACTOGRAFI FIBER MRI KUANTITATIF DALAM LEUKOARAIOSIS: ANALISIS BAHAN PUTIH CEREBRAL LESIONAL DAN BUKAN LESIONAL

Latar Belakang: Leukoaraiosis pertama kali digambarkan sebagai kawasan hipodensiti pada pengimejan CT dan pada MRI konvensional, ia adalah hiperintense yang kelihatan pada kedua-dua berwajaran T2 dan penyongsangan pengecilan cecair (FLAIR). Kedua-duanya mungkin mewakili pelbagai patologi yang berbeza, hanya sebahagian daripadanya dikaitkan dengan kemusnahan tisu dan disfungsi neuron. Pengimejan tensor resapan (DTI) Pengimejan MR adalah berdasarkan pergerakan molekul air dalam tisu biologi. Pengimejan DT digunakan secara meluas untuk mengkaji struktur mikro dalam otak, muskuloskeletal dan saraf tunjang. Sebarang perubahan pada tisu akan menjejaskan pergerakan molekul air yang memberikan maklumat mikroskopi mengenai tisu yang membolehkan pengesanan keabnormalan pada peringkat awal. Ciri-ciri tersendiri pengimejan DT ialah trakografi gentian yang memberikan penilaian kuantitatif dan kualitatif gentian saraf di dalam otak. Kajian ini membandingkan nilai Indeks ketumpatan ADC, FA dan Fiber antara leukoaraiosis dan subjek kawalan normal menggunakan sistem MRI 3T.

Metodologi: Kajian retrospektif telah dijalankan di Hospital USM, Kota Bharu. Kajian ini melibatkan 34 subjek sihat dan 34 pesakit Leukoaraiosis. Kesemua mereka menjalani pengimejan resonans magnetik (MRI) di mana imej kemudiannya dipraproses dan menjalani analisis tahap pertama menggunakan perisian trakografi. Analisis tahap kedua kemudiannya dilakukan oleh kawasan menarik (ROI) yang dilukis secara manual di rantau leukoaraiosis dan untuk membandingkan nilai kawasan faedah dalam subjek kawalan biasa. Untuk hasil yang berkesan, perbandingan padanan umur dengan kawasan otak yang sama telah dipilih untuk

kedua-dua subjek kawalan dan pesakit Leukoaraiosis. FA, ADC, isipadu gentian dan voxel dijana secara automatik oleh perisian. Analisis statistik menggunakan ujian-T berpasangan.

Keputusan: Purata nilai FA dalam kumpulan kawalan dan leukoaraiosis masing-masing ialah 0.393 (±0.039) dan 0.333 (±0.058). Purata nilai ADC dalam kumpulan kawalan dan leukoaraiosis masing-masing ialah 0.782 (±0.036) dan 0.823 (±0.068). Purata nilai FDI dalam kumpulan kawalan dan leukoaraiosis masing-masing ialah 1.341 (±0.310) dan 1.062 (±0.240). Perbezaannya adalah signifikan secara statistik dalam nilai FA, ADC dan FDI antara dua kumpulan.

Kesimpulan: Kajian ini mendapati terdapat perbezaan nilai parametrik trakografi MRI antara Leukoaraiosis dan subjek kawalan. Keupayaan trakografi gentian berasaskan MRI untuk menggambarkan laluan gentian bahan putih otak secara bukan invasif telah menimbulkan kemungkinan untuk aplikasi klinikal dan menawarkan potensi yang digunakan untuk pengesanan awal integriti bahan putih, penilaian keterukan penyakit dan perkembangan pada masa hadapan.

Kata kunci: *Fractional anisotrophy (FA); Apparent diffusion coefficient (ADC); fiber density index; Leukoaraiosis; DTI Tractography*

QUANTITATIVE MRI FIBRE TRACTOGRAPHY IN LEUKOARAIOSIS: ANALYSIS OF LESIONAL AND NON-LESIONAL CEREBRAL WHITE MATTER

ABSTRACT

Background: Leukoaraiosis was first described as area of hypodensity on CT imaging and on conventional MRI, it is an appear hyperintense on both T2-weighted and fluid attenuation recovery inversion (FLAIR). Both may represent a variety of different pathologies, only some of which are associated with tissue destruction and neuronal disfunction. Diffusion tensor imaging (DTI) MR imaging was based on movement of water molecules in biology tissue. DT imaging was widely used to study microstructural in brain, musculoskeletal and spinal cord. Any change in tissue will affect the water molecules movement that provide microscopy information regarding the tissue which allow detection of abnormalities in early stage. The distinctive features of DT imaging are fiber tractography which give quantitative and qualitative assesment of the nerve fiber in the brain. This study compares the ADC, FA and Fiber density Index value between leukoaraiosis and normal control subjects using 3T MRI system.

Methodology: A retrospective study was conducted in Hospital USM, Kota Bharu for 36month duration. This study involves age-matched 34 healthy subjects and 34 Leukoaraiosis patients. All of them had undergone magnetic resonance imaging (MRI) where images were then post-processed and underwent first level analysis to meet the optimum sequence and DTI parameter. Second level analysis were then performed using tractography software by manually drawed region of interest (ROI) at leukoaraiosis area and to compare the parametric value with normal appearing white matter subjects at the same region. For effective result, age-matched comparison with same area of brain regions were chosen for both control subjects and

leukoaraiosis patients. FA, ADC, fiber volume and voxel were generate automatically by the software. Statistical analysis was using paired T-test to compare value of both group.

Results: The mean of FA value in control and leukoaraiosis group were 0.393 (±0.039) and 0.333 (±0.058) respectively. The mean of ADC value in control and leukoaraiosis group were 0.782 (±0.036) and 0.823 (±0.068) respectively. The mean of FDI value in control and leukoaraiosis group were 1.341 (±0.310) and 1.062 (±0.240) respectively. The different was statistically significant in FA, ADC and FDI value between two group.

Conclusion: This study found that there is a statistically difference mean value of FA, ADC and FDI between leukoaraiosis and normal appearing white matter control subject. The ability of MRI- based fiber tractography to delineate the white matter fibre pathways of the brain non- invasively has raised possibilities for clinical applications and offers potential used for early detection of white matter integrity, evaluation of white matter severity and progression in the future.

Keywords: *Fractional anisotrophy (FA); Apparent diffusion coefficient (ADC); fiber density index; Leukoaraiosis; DTI Tractography.*

CHAPTER 1: BACKGROUND

1.1 Introduction

Leukoaraiosis is a common finding on MRI and has been strongly associated with risk of incident stroke and dementia. There is increasing evidence from neuroimaging to support that leukoaraiosis may be an independent predictor of stroke outcomes, too. Despite more than 15 years of extensive study, there remain significant unanswered questions regarding the pathogenesis of these very common lesions. Leukoaraiosis was first described as area of hypodensity on CT imaging and on conventional MRI, it is an appear hyperintense on both T2-weighted and fluid attenuation recovery inversion (FLAIR). However, both may represent a variety of different pathologies, only some of which are associated with tissue destruction and neuronal disfunction.

The development of diffusion tensor imaging (TI) has opened the possibility for investigation structural and anatomical features of white matter noninvasively. It detects and measures mobility of water molecules in biological cells. It offers quantitative information on micromolecular and microstructural changes in biological tissue by providing the scalar value and anisotropy indices for analysis. However, the main drawback is it only provide 2D scaling data involve placing the regions of interest on DTI maps. The more advanced technique is by reconstruct the DTI data on 3D mapping called tractography. It is creating the geometric model that reflect the large scales structure of fiber bundles. The primary advantage of tractography compared with regional analysis of scalar metric is the integration of data across an entire white matter tract as well as diffusivity properties sampled along the fiber bundle.

Many of the DTI tractography study was perform using 1.5-Tesla MRI (Taib NH et al 2014) (Matsuo K et al 2008)(Kim JE et al 2014). When comparing 1.5T with 3T, field strength is the biggest difference. Three tesla systems allow for higher SNR as well as increased image resolution capabilities, which is crucial for MR imaging. According to (Andrew L Alexander et al 2006) DTI studies at 3.0 T MRI using parallel imaging will provide significantly improved DTI measurements relative to studies at 1.5 Tesla MRI. Subjective image quality of FA maps at 3.0 T scanner is superior to that at 1.5 T with improved SNR (Kido Aki et al 2010). Therefore, our aim is to study the diffusion tensor tractography parametric value in leukoaraiosis patient using 3-Tesla MRI machine.

Most of tractography study was on other clinical disease such as stroke, dementia, multiple sclerosis and schizophrenia. Till now no published DT Tractography data study for leukoaraiosis using 3T MRI machine yet. So, based on parametric value changes seen in leukoaraiosis patient between normal appearing white matter, our aim this study could provide data for risk prediction and early detection of related disease such as stroke, dementia or cognitive impairment. Futhermore, it will help in assessing the severity of the disease as well as monitoring of disease progression.

1.2 Objectives

1.2.1 General objective

To study the diffusion tensor tractography parametric values in leukoaraiosis patients using 3T MRI fiber tractography.

1.2.2 Specific objectives

1. To compare tractography fractional anisotropy (FA) value between leukoaraiosis and normal appearing brain white matter of control subject.
2. To compare tractography apparent diffusion coefficient (ADC) value between leukoaraiosis and normal appearing brain white matter of control subject.
3. To compare fiber density index (FDI) value between leukoaraiosis and normal appearing brain white matter of control subject.

1.3 Research Questions

Is there any different between fractional anisotropy (FA), apparent diffusion coefficient (ADC) and fiber density index (FDI) value in leukoaraoisis compare with normal normal appearing white matter subject?

CHAPTER 2: LITERATURE REVIEW

2.1 Leukoaraiosis

The terms leukoaraiosis, cerebral white matter disease and cerebral small vessel disease are often used synonymously. They refer to the presence of white matter lesions in the brain, which are caused by chronic small vessel ischemia, blood brain barrier dysfunction or, most likely, a combination of these two factors. (Hachinski V *et al* 2019). Leukoaraiosis is a common neuroimaging finding that is associated with stroke outcome as well as stroke incidence (Wiszniewska *et al* 2000, Guo Y *et al* 2019). In Greek leuko means white and araiosis means rarified. The term therefore indicates the rarefaction, or reduced density, of the white matter (Grueter BE *et al* 2012). Leukoaraiosis is a radiological diagnosis obtained with brain imaging using either CT or MRI. White matter changes appear as low attenuation areas on CT and as areas with high signal on MRI. However, MRI is more sensitive than CT in the detection of small lesions while larger lesions are detected equally well with either imaging method. On MRI areas of leukoaraiosis usually appear hypointense in T1-weighted image and hyperintese in T2-weighted and FLAIR image of the white matter area (Jones DK *et al* 2001).

2.2 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) has rapidly evolved as it provides microstructural information of then tissue mostly in central nervous system. Diffusion tensor imaging had been applied to detect brain ischemia since in early 1990s as the water diffusion reduced in ischaemic event DTI had been applied to study organization of structure in spinal cord, kidney, and heart (Le Bihan D *et al* 2001).

The movement of the water molecules was constituted with arrow called eigenvector mathematically. If the water molecules movement was similar in multidirectional called

isotopic with same length of arrow, eigenvalue(λ) The contrary to it was anisotropic diffusion which the molecule motion was in on direction as impeded by walls of cells such as in nerve fibre. The eigenvalue (λ') is longer compared to other value (λ2 & λ3) in anisotropy tissue. Fractional anisotropy (FA) exhibits the degree of tissue anisotropy. The value is O for erfect isotropy to 1 for total anisotropy. Decreased of FA in highly anisotropy tissue as caused by distruption of well-organized cellular architecture by physiology or pathologic process (Levit *et al* 2018).

2.3 Apparent diffusion coeficient(ADC), Fractional anisotropy(FA) & Fiber Density Index (FDI).

The main apparatus in DT imaging are apparent diffusion coefficient (ADC) and fractional anisotropy (FA). As mentioned previously, its portrays the biological movement of the water molecules in the tissue. Therefore, it's given a value which was in the normal or diseased tissue. Apparent diffusion coefficient reflects the water molecules movement along the direction of the diffusion sensitizing gradient. The greater of water diffusion, the higher ADC value and vice versa. Reduced in ADC giving specific diagnosis in acute cerebral ischemia such as in status epilepticus (Mukherjee *et al*, 2008). Fractional anisotropy (FA) exhibits the degree of tissue anisotropy. Decreased of FA in highly anisotropy tissue as caused by distruption of well-organized cellular architecture by physiology or pathologic process (Budzik *et al*, 2014).

Fiber Density Index (FDI) is a quantitative index of continuous fiber volume traversing a selected ROI (voxel) on fiber tractography image. It was measured by number of fiber volume through ROI divided with area of ROI in certain voxel. FDI value will depend upon factors such as the spatial resolution of the image acquisition and parameters of postprocessing (FA cutoff and angulation threshold). The volume of the gray matter decreases linearly by about

5% per decade with age, has been replicated in several studies (Pareek v et al., 2018, Kubicki M et al., 2005). There is a significant reduction of fiber volume in diffused axonal injury due to loss of white matter integrity (Wang JY et al. 2008).

2.4 DTI Tractography – technical aspect and clinically significant.

By combining the directional information and magnitude of anisotropic diffusion of the individual voxels, the course of white matter tracts can be reconstructed (Huisman, 2010). This technique, which is also known as tractography relies on the assumption that voxels with a similar orientation of their principal anisotropic diffusion direction is likely part of the same white matter tract. Complex crossings of white matter tracts within one voxel can be resolved by increasing the number of diffusion-encoding gradients and a powerful postprocessing mathematical algorithms. These fibers are then color-coded to differentiate the type and different direction. The processing of tractography can be divided into three main stages, namely seeding, propagation and termination. Seeding consists of specifying the points from which to draw the bundles of fibres. One of the most common methodologies is to identify Regions of Interest (ROIs), and to position one or more seeds in each ROI voxel. The ROIs can be manually drawn or extracted from other MRI modalities (Soares et al., 2013). The fibers are produced progressively during the propagation stage. Fiber tracking can be achieved by two different algorithm categories: deterministic and probabilistic (Descoteaux et al., 2009). Deterministic tractography aims to model the data and can be thought as generating/reconstructing one fiber from each seed. Probabilistic methods consider the estimation's uncertainty, which results in probability maps reflecting the possibility that a voxel is part of a fiber and provides the multiple potential fiber directions emanating from each seed. The last tractography step is termination of the fiber tracking procedure based on termination criteria. The aim of this criteria is to avoid propagating the fibers in voxels where robustness

of the vectorial field is guaranteed. The common termination criteria are minimum FA thresholds and turning angle threshold (Soares et al., 2013). These termination criteria can be set up in the DTI fiber tractography software.

There are several software tools used in literature for DTI image analysis and fiber tractography as reviewed by Soares et al. (2013). The example of DTI processing software and its utilization are BrainVoyager QX (Frost MA et al, 2012), 3D Slicer (Pieper et al., 2004), ExploreDTI (Leemans et al., 2009) and Freesurfer (Fischl et al, 2012). In our study we are using Intellispace Portal Version 10 software which is better and high compatibility with MRI Philips 3T machine.

DTI technique has been vast studying various diseases, such as stroke (Van Der Aa et al 2011), multiple sclerosis (Sbardella et al 2013), brain tumor (Raffa G et al 2016), Alzheimer's disease and Schizophrenia. Besides, diffusion MR Tractography has recently emerged as potentially valuable non-invasive clinical tool for presurgical planning and intraoperative imaging guided navigation in the operating room aided by knowing the proximity and relative position of the tumor to the adjacent WM tracts (Panesar SS et al 2019). MR Tractography can be used to virtually dissect functionally critical white matter tracts, such as the corticospinal tract and the arcuate fasciculus (AF), enabling the neurosurgeon to identify and preserve the tract during resection.

2.5 DTI imaging and Tractography in Leukoaraiosis.

Leukoaraiosis is a common finding in stroke patients and has been strongly associated with risk of incident stroke and dementia. Despite the large number of studies, the cause and pathogenesis of leukoaraiosis remain unknown. Preventing leukoaraiosis may reduce not only

the population health burden from cognitive impairment but may also reduce neurological disability by improving outcomes from ischemic stroke.

Recently, DTI has become established as a tool for brain imaging. DTI is a non-invasive imaging technique for investigating changes in white matter integrity (Davis SW et al., 2009). It provides quantitative information on the microstructural integrity of the white matter.
The application of DTI in basic clinical research has been employed in research on assessment of white matter abnormalities particularly leukoaraiosis (O'Sullivan et al., 2004).
Based on post processing DTI data, it also allows for visualization of neuron fibres through a specific technique called fibre tractography. Feasibility of acquiring DTI data and constructing fiber tractography for leukoaraiosis study was investigated using 1.5T MRI. Results obtained showed capability to distinguish leukoaraiosis from normal white matter and fiber tractography exhibits an obvious discontinuity of several nerve fibre tracts at the area of leukoaraiosis (Taib NH et al 2014).

Main parameter for quantitative analysis of DTI tractography was apparent diffusion coefficient (ADC), fractional anisotropy (FA) and fiber density index (FDI). Diffusion tensor MRI can provide information about the integrity of axonal tracts. In leukoaraiosis, loss of anisotropy (FA) and increase in ADC occurs because of axonal loss and gliosis. Therefore, exhibit reduced of FA value in leukoaraiosis area compared to normal brain in all lobes (Taib NH et al 2014). The more severe the leukoaraiosis was, the higher the ADC values of the leukoaraiotic regions became (Helenius J et al 2002). DTI has also shown decreased FA values in the affected parietooccipital white matter even in those with near-normal conventional imaging (Trivedi R et al 2006). All of these significant changes may help in earlier detection and more accurate assessment of microstructural brain damage (Etherton et al 2016). The

extent and spatial pattern of the loss of fractional anisotropy may correlate with clinical parameters, particularly the degree of cognitive impairment. By deriving maps of the orientation of white matter tracts, it may be possible to correlate disruption of tracts with loss of specific cortical functions and the pattern of any cognitive impairment. Monitoring the degree of reduction in fractional anisotropy and the increase in trace may allow monitoring of disease progression.

Therefore, in this study, FA, ADC and FDI will be used as DTI Tractography parameter to investigate the changes occurring in the leukoaraiosis patient. Then comparison will be made with normal appearing white matter in healthy subject using 3T MRI machine.

CHAPTER 3: METHODOLOGY

3.1 Study Design

Retrospective case study.

3.2 Study Location and Duration

Hospital Universiti Sains Malaysia.

Study was conducted for a period of 36 month from June 2017 until June 2020.

3.3 Study Population and Sample

Reference population: Individual who are fulfilled in inclusion criteria. Study area in MRI room at Hospital Universiti Sains Malaysia Kubang Kerian, Kelantan.

3.4 Inclusion and Exclusion Criteria for leukoaraiosis

Inclusion criteria for Leukoaraiosis

1. Age between 35-60 years old
2. Abnormal appearing white matter on MRI (Leukoaraiosis)
3. Underwent MRI brain sequence (T2WI/FLAIR and DTI)

Exclusion criteria for Leukoaraiosis

1. Presence of malignant features on MRI
2. Presence of congenital brain structure abnormalities on MRI.
3. Presence of post brain surgery changes.
4. Incomplete MRI brain images or sequence
5. Poor MRI image quality with susceptibility effect or motion.

3.5 Inclusion and Exclusion Criteria for normal appearing white matter subject.

Inclusion criteria for healthy subjects

1. Age between 35-60 years old
2. No abnormal white matter lesion on MRI
3. Underwent MRI brain sequence ((T2WI/FLAIR and DTI)

Exclusion criteria for healthy subjects

1. Presence of malignant features on MRI
2. Presence of congenital brain structure abnormalities on MRI.
3. Presence of post brain surgery changes.
4. Incomplete MRI brain images or sequence
5. Poor MRI image quality with susceptibility effect or motion.

3.6 Sample Size Calculation

Objective 1

Objective: To compare tractography fractional anisotropy (FA) value between leukoaraiosis and normal appearing brain white matter of control subject.

Method: We applied the 2 means - Hypothesis Testing using Sample Size Calculator by Arifin, W. N. (2021) to estimate our sample size. Details of the formula is listed below:

- Standard deviation (G): 0.20 (Taib *et al*, (2012))
- Expected difference: 0.19
- Significance level (a): 0.05 Two-tailed
- Power (1 - B): 80 %
- Expected dropout rate: 10 %
- Sample size, n = 18
- Sample size (with 10% dropout), drop = 20
- Total sample size – 40

Objective 2

Objective: To compare tractography apparent diffusion coefficient (ADC) value between leukoaraiosis and normal appearing brain white matter of control subject.

Method: We applied the 2 means - Hypothesis Testing using Sample Size Calculator by Arifin, W. N. (2021) to estimate our sample size. Details of the formula is listed below:

- Standard deviation (G): 50 (Taib *et al*, (2012))
- Expected difference: 35
- Significance level (a): 0.05 Two-tailed
- Power (1 - ß): 80 %

- Expected dropout rate: 10 %
- Sample size, n = 33
- Sample size (with 10% dropout), drop = 37
- Total sample size – 78

Objective 3

Objective: To compare tractography Fiber density Index (FDI) value between Leukoaraiosis and normal-appearing brain white matter of control subject.

Method: We applied the 2 means - Hypothesis Testing using Sample Size Calculator by Arifin, W. N. (2021) to estimate our sample size. Details of the formula is listed below:

- Standard deviation (G): 16.2 (Andreisek G *et al* 2010)
- Expected difference: 15
- Significance level (a): 0.05 Two-tailed
- Power (1 - B): 80 %
- Expected dropout rate: 10 %
- Sample size, n = 18
- Sample size (with 10% dropout), drop = 20
- Total sample size – 48

Final Sample Size

The final total sample size estimated for our study is **78 samples.**

3.7 Research Tools

1. MRI machine - Phillips 3 Tesla Achieva MR scanner, Best, The Netherland 2016 for brain image acquisition.
2. Intellispace Portal Version 10, Best, The Netherland 2013 - Platform for tractography reconstruction and analysis.
3. PACS UV (Picture Archiving and Communication System)

3.8 Variable Definition

i. Diffusion tensor tractography (DTT) - is a non-invasive imaging technique that provides quantitative information about the directionality (anisotropy) and magnitude (diffusivity) of major white matter (WM) tracts in 6-256 dimensions of the human brain.

ii. Leukoaroisis - abnormal radiological appearance or signal changes of the brain white matter. It appears hypodense on CT and on MRI will appear high signal on T2 weighted and FLAIR images. It can be bilateral or unilateral, patchy or diffused. It divided into periventricular and deep/subcortical white matter lesion.

iii. DTI Tractography parameters:

- *Fractional anisotropy (FA)* - is a normalized ratio of diffusion directionality. It shows directionality of molecular displacement by diffusion and vary between 0 (isotropic diffusion) and 1 (infinite anisotropic diffusion).
- *Apparent diffusion coefficient (ADC)* - are calculated automatically by the software and then displayed as a parametric map that reflects the degree of diffusion of water molecules through different tissues.
- *Fiber Density Index (FDI)* - Is quantitative index of continuous fiber path traversing an ROI on fiber tractography images. It was measured by volume number of fiber path divided with an area of ROI in a certain voxel.

3.8 Data Collection

3.8.1 Subject recruitment

Patients which underwent MRI Brain at Hospital Universiti Sains Malaysia with abnormal appearance of white matter which showed hyperintense on both T2-weighted and fluid attenuation recovery inversion (FLAIR). Risk and benefit were explained to the patient thoroughly. Subject's information i.e., age, gender, anisotraphic parameter value were recorded in the data collection sheet.

3.8.2 Image Acquisition

In this study, imaging studies were performed with a 3T MRI system (Achieva 3.2.3.4, Philips) using a 32-channel receiver head coil. Conventional MR images were acquired according to the standard brain protocol in our hospital, the selected restrospective image were acquired which involved the following sequence:

i. T2 axial, TE /TR 80/3000ms, matrix 512 x 512; FOV 230.0x 230.0, NEX 1.0; slice spacing 1.0mm, slice thickness 5.0mm, flip angle 90
ii. FLAIR axial, TE /TR /TI 125/ 11000/2800ms, matrix 512 x 512; FOV 230.0x 230.0, NEX 1.0; slice spacing 1.0mm, slice thickness 5.0mm, flip angle 90
iii. DTI 32, TE/TR 72/10000ms.

The parameters for the DTI sequence were the repetition time (TR) 5144 ms, echo time (TE) 92 ms, field-of view (FOV) 240 mm, matrix 96X96, 3 averages, slice/gap 2.5/0.9 mm, flip angle 70, voxel dimension $1.8 \times 1.8 \times 3.0$ mm^3, b-value 0 and 400 s/mm^2, and 20 diffusion gradient orientations. Data were acquired using Stejskal-Tanner sequence with single shot echo planar imaging (SS-EPI). Parallel imaging was performed using sensitivity-encoding (SENSE) algorithm,` with an acceleration factor of 2 to reduce to reduced total acquisition time and magnetic susceptibility artifact.

3.8.3 Image processing and quantitative measurement

Image were retrieved from Picture archiving and communication system (PACS UV). The analysis was performed using IntelliSpace Portal Version 10 worstation. The raw data were selected. Each of these images was co-registered with baseline image to correct the eddy-current geometric distortion.

Fiber tractography was selected from tools option and raw data of diffusion image was processed by the software (IntelliSpace Portal Version 10, Best, The Netherland 2013). The diffusion image was merge with T2 and FLAIR axial view to delineate the anatomical area of abnormal high signal of white matter area (Figure 1). Freehand contour type was selected and circular region of interest (ROIs) was manually drawn (Hakulinen U et al 2021) on pre-processed T2/FLAIR images for anatomical landmarks in the brain (Figure 2). The ROI was located in an axial plane that best represented of the lesion. The single seed of ROIs (de Luis-Garcia R *et al* 2010) were drawn independently with the same brain landmark/region between age-matched leukoaraiosis and normal appearing white matter subject. It was evaluated by principal investigator and a senior radiologist (more than 10 years' experience). Fiber tracking was performed using fiber assignment by continuous tracking (FACT). The fibre tractography maps was generated automatically with FA threshold more than 0.2 with maximum turning angle of 30 (Figure 3, Figure 4). FA, ADC, Fiber volume and voxel are generated automatically by the software. For each leukoaraosis and normal appearing white matter subject subject, the measures of FA, ADC and FDI are calculated.

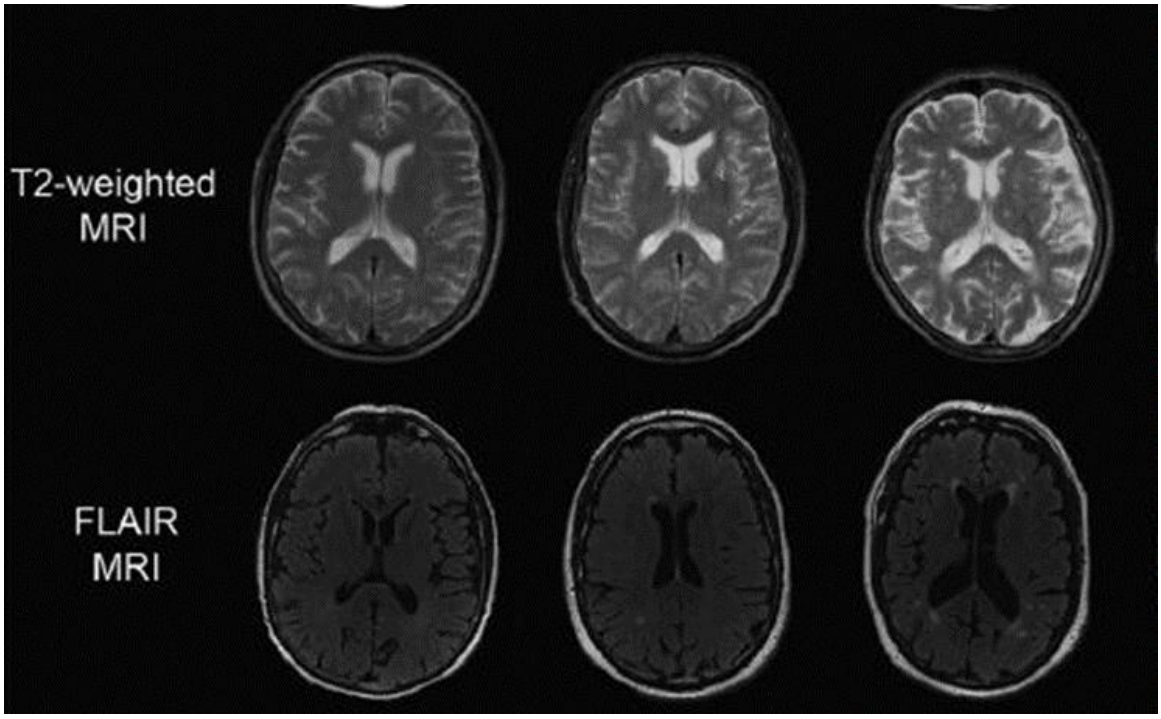

Figure 1: Leukoaraiosis on T2WI and FLAIR sequence appear hyperintense on MRI The leukoaraiosis may represent as appear focal, diffused, single or confluence lesion.

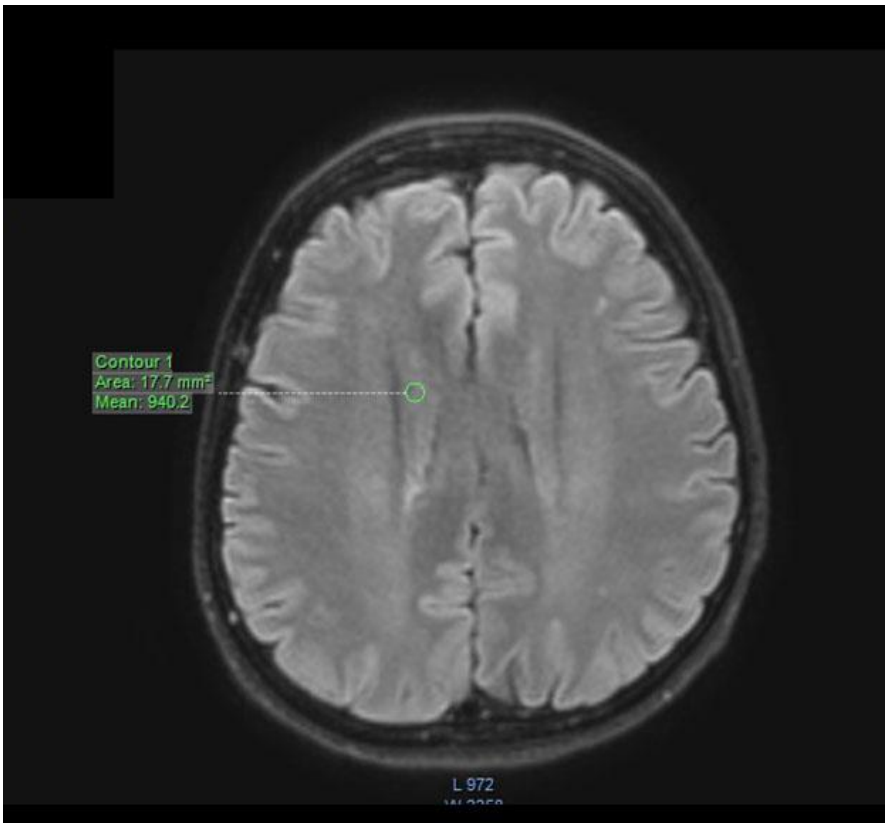

Figure 2: An example of circular single-ROI placement on white matter region. This ROI were identified on FLAIR image.

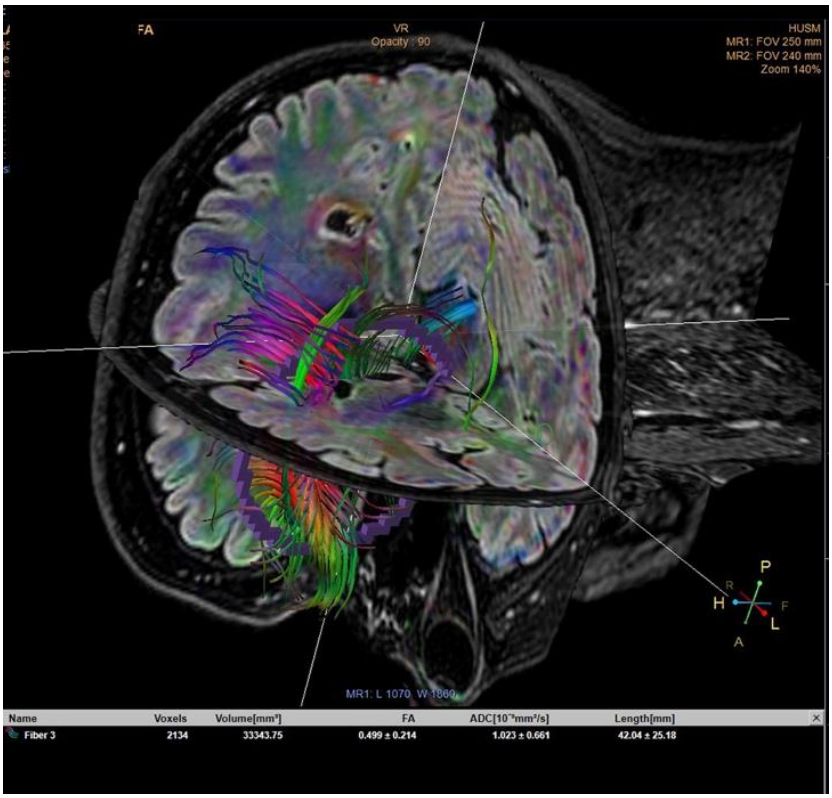

Figure 3: Diffusion tensor tractography with color maps.

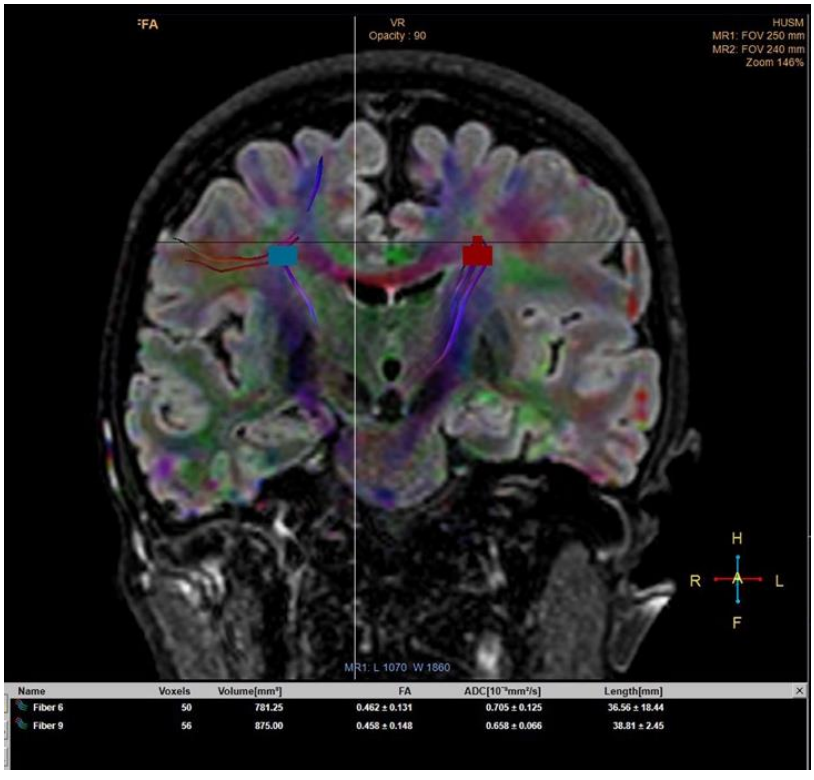

Figure 4: Automated parametric value obtained on selected ROI placement via tractography map.

3.9 Statistical Analysis & Hypothesis

1. All quantitative data were analysed using Microsoft® Office Excel and Statistical Product and Service Solutions (SPSS) for Windows, SPSS Inc.© (version 26.0, SPSS Inc., Chicago, IL, USA).

2. Histogram and chart will be used to present the numerical and categorical data respectively. All categorical data were presented in frequency and percentage, while the numerical data were presented in mean and standard deviation.

3. The comparison study between two groups were tested using the independent t-test when appropriate. It was employed to perform the statistical analysis for comparison of ADC, FA and FDI values for each region of interest between normal appearing white matter control subject and leukoaraiosis subjects.

4. Normality tests using Kolmogorov-Smirnov, Shapiro-Wilk and histogram diagrams found our data to be normally distributed within each group.

4. The P level of less than 0.05 was considered statistically significant

Null hypothesis

There will be no difference in tractography anisotraphic value of leukoaraiosis patients and normal appearing white matter subjects.

3.10 Confidentiality and Privacy

All data were anonymous and entered into SPSS software. Only research team members can access the data. Data presented as grouped data and not identify the responders individually.

3.11 Ethical Consideration

1. Ethical approval

   The research has received ethical approval Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM code: USM/JEPeM/20080424) which complies with the Declaration of Helsinki (Appendix E).

2. Informed consent/Assent Process

   Informed consent from previous study includes this study. Approval to use the data taken from the hospital's director.

3. Subject vulnerability

   Not applicable.

4. Declaration of absence of conflict of interest

   The investigators declare they have no conflict of interest.

5. Community sensitivities and benefits

There was no issue arise that affect community sensitivities. The collected data will enrich the knowledge regarding factor affecting white matter neuronal pathway and the relevant of MRI tractography study.

To our knowledge this study able to evaluate the need of post processing tractography study in Leukoaraiosis. The reference values will guide the physician to effectively detect early neuronal fibre changes of brain parenchyma related to white matter disease.

6. Publication policy

No personal information will be disclosed as the subjects will not be identified individually when the findings of the study are published.