QUANTITATIVE ANALYSIS OF LEUKOARAIOSIS USING DIFFUSION TENSOR IMAGING: A RETROSPECTIVE STUDY

SITI NOR FATIHAH BINTI SALBINIH @ SALBINI

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by

SITI NOR FATIHAH BINTI SALBINIH @ SALBINI

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"In the name of Allah, The Most Gracious, The Most Merciful"

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D	Diffusion coefficient (mm^2/s)
D’	New tensor
G	Amplitude
S	Signal intensity with diffusion-weighting
S_0	Signal intensity without diffusion-weighting
Γ	Gyromagnetic ratio (MHz/T)
Δ	Duration of diffusion pulse (ms)
Δ	Time between the two sensitizing gradient lobes (ms)
$\mathbf{v}_1$, $\mathbf{v}_2$, $\mathbf{v}_3$	Eigenvectors that correspond to each λ_1, λ_2, and λ_3
$D_\perp$	Radial diffusivity (mm^2/s)
b	b-value (s/mm^2)
λ_1, λ_2, λ_3	Eigenvalues of the diffusion tensor

LIST OF ABBREVIATIONS

AD	Axial Diffusivity
ADC	Apparent Diffusion Coefficient
AP	Anterior-Posterior
CSF	Cerebrospinal fluid
CSVD	Cerebral small vessel disease
CV	Coefficient of variation
DTI	Diffusion Tensor Imaging
FA	Fractional Anisotropy
FLAIR	Fluid Attenuated Inversion Recovery
FOV	Field of view
fWM	Frontal white matter
LA	Leukoaraiosis
LNR	Lesion-to-Normal Ratio
MD	Medial Diffusivity
MRI	Magnetic resonance imaging
NAWM	Normal Appearing White Matter
oWM	Occipital white matter
PD	Proton density
PGSE	Pulsed Gradient Spin Echo
RD	Radial Diffusivity
RGB	Red-Green-Blue
RL	Right-Left
ROI	Region of interest
SD	Standard deviation

SI	Superior-Inferior
T1	Longitudinal relaxation time
T2	Transversal relaxation time
TE	Echo time
TR	Repetition time
WM	White matter
WMC	White matter changes
WMD	White matter disease
WMH	White matter hyperintensity
WML	White matter lesion

ANALISIS KUANTITATIF LEUKOARAIOSIS MENGGUNAKAN PENGIMEJAN TENSOR DIFUSI: KAJIAN RETROSPEKTIF

ABSTRAK

Kajian ini merupakan kajian retrospektif yang memfokuskan kepada analisis kuantitatif leukoaraiosis dengan menggunakan pengimejan tensor difusi. Leukoaraiosis (LA) ialah penampilan patologi jirim putih dalam otak yang kelihatan sebagai jirim putih hiperintensiti pada imbasan otak MRI imej berwajaran T2 atau imbasan CT. Imej yang digunakan dalam kajian ini terdiri daripada imej DTI otak manusia yang diperolehi menggunakan sistem GE 1.5 T MRI yang terdapat di Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia dari tahun 2012 hingga 2013. Empat objektif utama yang digariskan dalam kajian ini telah dicapai. Imej otak 43 subjek dengan leukoaraiosis diambil dari sistem USM PACS. Empat jenis analisis telah dilakukan dalam kajian ini. Pertama adalah analisis demografi untuk mendapatkan gambaran keseluruhan mengenai jantina, umur, bilangan tompokan lesi dan saiz tompokan lesi. Kemudian, perbandingan nilai DTI genu dan splenium dalam corpus callosum antara subjek dengan leukoaraiosis dan tanpa leukoaraiosis telah dilakukan. Didapati bahawa nilai AD ($p = 0.70$; $p = 0.46$), RD ($p = 0.71$; $p = 0.33$), MD ($p = 0.67$; $p = 0.34$) dan FA ($p = 0.67$; $p = 0.34$), di genu dan splenium masing-masing pada subjek dengan leukoaraiosis tidak berbeza secara signifikan daripada yang tidak mempunyai leukoaraiosis di kedua-dua kawasan tersebut. Seterusnya, kajian mengenai kebolehubahan intra dan antara subjek telah dilakukan bagi mereka yang mempunyai 10 tompokan lesi dan ke atas (≥ 10), ($n = 8$). Enam subjek menunjukkan perbezaan FA dan LNR yang tidak signifikan manakala dua subjek menunjukkan perbezaan FA dan LNR yang signifikan dalam subjek. Terdapat variasi

nilai FA dan LNR di seluruh saiz tompokan lesi dalam subjek di mana tompokan lesi terkecil mempunyai nilai FA lebih rendah dan LNR lebih tinggi dan tompokan lesi terbesar mempunyai sedikit perbezaan antara nilai FA dan LNR. Untuk kebolehubahan antara subjek, subjek mempunyai perbezaan FA dan LNR yang ketara. Nilai FA dan LNR berbeza mengikut jumlah tompokan lesi. Akhir sekali, perbandingan FA dan LNR dilakukan untuk menilai keterukan perubahan tisu leukoaraiosis antara subjek yang mempunyai tompokan lesi yang besar dan sedikit bilangannya dan mereka yang mempunyai banyak tompokan kecil dan banyak bilangannya tetapi mempunyai jumlah isipadu tompokan lesi yang sama. Didapati bahawa subjek dengan banyak tompokan lesi kecil menunjukkan nilai FA yang lebih rendah dan nilai LNR yang lebih tinggi berbanding dengan mereka yang mempunyai sedikit tompokan lesi yang besar. Ini menunjukkan bahawa mereka yang mempunyai banyak tompokan lesi kecil mempunyai kerosakan tisu yang lebih teruk daripada mereka yang mempunyai sedikit tompokan lesi besar.

QUANTITATIVE ANALYSIS OF LEUKOARAIOSIS USING DIFFUSION TENSOR IMAGING: A RETROSPECTIVE STUDY

ABSTRACT

This research is a retrospective study that focused on quantitative analysis leukoaraiosis with the application of diffusion tensor imaging (DTI). Leukoaraiosis (LA) is a pathological appearance of white matter in the brain which appearing as hyperintensity white matter on T2-weighted image MRI brain scans or CT scans.

The images used in this study consisted of DTI images of human brain was obtained using GE 1.5 T MRI system available in Advanced Medical and Dental Institute, Universiti Sains Malaysia from year 2012 to 2013. Four main objectives outlined in the study have achieved. Brain images of 43 subjects with leukoaraiosis retrieved from USM PACS system. Four types of analysis have been performed in this study.

First is the demographic analysis to have an overview on the gender, age, number of lesion spots and lesion size. This demographic analysis showed that there were more female subject participated in this study. The most subject were aged from 50 to 59 years old.

Then, comparison of DTI values of genu and splenium in corpus callosum between subjects with and without leukoaraiosis was performed. It was found that AD ($p = 0.70$; $p = 0.46$), RD ($p = 0.71$; $p = 0.33$), MD ($p = 0.67$; $p = 0.34$) and FA values ($p = 0.67$; $p = 0.34$) of genu and splenium in subjects with leukoaraiosis does not differ significantly from those without leukoaraiosis in the two areas.

Next, investigation on the intra- and inter-subject variability has been performed for those who have 10 lesion spots and above (≥ 10), ($n = 8$). Six subjects

showed insignificant differences of FA and LNR while two subjects showed significant differences of FA and LNR within subjects. There are variation of FA and LNR values across lesion sizes within subjects where the smallest lesion got lower FA and higher LNR values and the biggest lesion got slightly difference between FA and LNR values. For inter-subject variability, the subjects have significant difference of FA and LNR. The FA and LNR values varies across the number of lesions.

Finally, comparison of FA and LNR values was performed to assess severity of leukoaraiosis tissue changes between subjects who has few big lesions and those with many lesions but having similar total lesion volume. It was found that subjects with many small lesion spots exhibit lower FA values and higher LNR values compared to those with few big lesion spots which indicates that those with many small lesion spots have more severe tissue damage than those few big lesion spots.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Magnetic resonance imaging (MRI) is an imaging modality that uses a very strong magnetic field. MRI can produce the detailed image from any part of the body as it uses the body's natural magnetic properties such as hydrogen nucleus, generally a single proton since its abundance in water and fat.

There are many advanced techniques in MRI. One of them is diffusion-weighted imaging (DWI). DWI is a technique which is sensitive to changes in the water molecules diffusion properties. The DWI is extended to diffusion tensor imaging (DTI) which utilizes a tissue water diffusion rate for image production (Ranzenberger & Snyder, 2019). The diffusion properties are described by apparent diffusion coefficient (ADC) maps for DWI which is the average of the tensor's eigenvalues. ADC maps are measured using fewer diffusion gradients than the tensor needed in clinical imaging since it was introduced to avoid the problems of non-Gaussian diffusion.

Meanwhile, mean diffusivity (MD) and fractional anisotropy (FA) represent the magnitude of the diffusion process (Soares at el., 2013). MD is referred to the total measure of diffusion in a voxel (O'Donnel & Westin, 2012), whereas FA represent the measure of the fraction of the tensor's magnitude, corresponding to the direction of anisotropic diffusion (Soares et al., 2013). Thus, MD and FA are the most widely used diffusion properties in aging research. Another two parameters also derived from DTI is radial diffusivity and axial diffusivity where these two parameters represent the axonal and myelination (Inano et al., 2011).

Besides providing quantitative information, another advantage of DTI is it allows visualisation of neuron fibres. DTI can derive the parameter based on the primary eigenvector of diffusion to obtain three-dimensional (3D) fiber bundles which is white matter pathway. This method called as fiber tractography (Soares et al. 2013). Weedden et al. (2012) analysed that this method projects 3D trajectories of fiber pathways and connection patterns between different brain systems in vivo.

1.2 Research problems

At Advanced Medical and Dental Institute Universiti Sains Malaysia (AMDI_USM), a pharmacological study on white matter lesion, specifically leukoaraiosis has been carried out. Researchers assessed the neuroprotective effects of tocotrienol vitamin E intake on leukoaraiosis. Evaluation of lesions progression was carried out using standard MRI protocols (Gopalan et al., 2014).

Feasibility of performing DTI scan and acquiring data was performed in other previous study (Mohd Taib et al., 2012). In the related previous work, Mohd Taib (2014) proposed a new method for characterizing leukoaraiosis namely lesion-to-normal appearing white matter ratio (LNR) (Mohd Taib et al., 2015; Mohd Taib, 2014).

Based on the previous study by Mohd Taib et al., (2020), they found that LNR is not associated with the lesion volume and suggested that degree of tissue damage is not associated with lesion size. Therefore, it is still unclear whether human brain with many small lesions and those with few big lesions has same degree of tissue damage. In the current study, which is based on convenience sampling, the available data will be further analysed to fill the research gap.

Diffusion magnetic resonance imaging will be used for the assessment of leukoaraiosis. The assessment includes characterization of leukoaraiosis at specific region using information derived from DTI maps. Leukoaraiosis is affected by various risk factors and mechanisms, it is assumed that severity of tissue destruction at different region, different age and different gender is not similar. Thus, there are various DTI values for different lesions across the brain.

The level of tissue damage is determined by FA and LNR values. This research will analyse the correlation between FA and LNR and also, to study white matter degree in brain with those few big lesions and many small lesions.

Evaluation of leukoaraiosis also consider the region where the leukoaraiosis is located. DTI indices values are measured at leukoaraiosis regions as well as normal appearing white matter (NAWM) at various regions in the brain. FA, MD, AD and RD values represent the microstructure of tissues.

1.3 Research questions

1.3(a) Is there any differences in DTI indices of the corpus callosum fibre bundles between subjects with and without leukoaraiosis in the frontal and occipital white matter areas?

1.3(b) In subjects who have many leukoaraiosis spots, to what extent is the variability between leukoaraiosis within and between subjects?

1.3(c) Do subjects with many small lesions and those with few big lesions has same degree of tissue damage?

1.4 Justification of study

The important contribution of this study is in the analysis of leukoaraiosis, specifically the degree of tissue damage for each lesion spot in individual subject. Measurement of lesion volume at each specific lesion spot for every subject which will be adopted in the present work will provide specific information on the degree of tissue damage at specific lesion spot as well as its distribution in the whole brain. By using the LNR index, which takes into account the normal white matter surrounding the lesion, also allows comparison to be made between different lesion spots either intra- or inter- individually.

1.5 Objectives

1.5.1 General objective

The aim of the study is to investigate the distribution of leukoaraiosis and the degree of tissue damage at each specific leukoaraiosis spot in individual subject.

1.5.2 Specific objectives

1.5.2(a) To determine statistically the distribution of subjects in this study in terms of the gender, age, number of lesions and lesion size.

1.5.2(b) To compare the DTI indices in the corpus callosum fibre bundles of subjects with and without leukoaraiosis of the frontal and occipital white matter areas.

1.5.2(c) To determine the intra- and inter-subject variability of leukoaraiosis among subjects who have 10 leukoaraiosis spots and above.

1.5.2(d) To compare severity of leukoaraiosis between FA and LNR of the leukoaraiosis between subjects who has few big lesions and those with many small lesions but having similar total lesion volume.

1.6 Scope of research

This study focused on the image-based patient-specific analysis of leukoaraiosis. The DTI parameter indices have been used to analyze the condition of the white matter and compare them with normal brain tissue. Investigation was also performed specifically on each lesion spot in the subject's brain and quantitative values was measured. In this study, FA and LNR were used to evaluate the degree of tissue destruction.

CHAPTER 2

LITERATURE REVIEW

2.1 White matter of the brain

Human brain is an important structure in the central nervous system. In the human brain, the cerebrum is the uppermost region of the central nervous system and it is the largest part of the human brain. The cerebrum is consisting left and right cerebral hemispheres, which are connected by the corpus callosum. It contains cerebral cortex (Zhang, 2019). The cerebral cortex has about 100 billion neurons. There are two main structures that form the cerebral cortex which is white matter and grey matter.

White matter is one of the central nervous system besides grey matter. Both are essential sections in the brain as well as spinal cord (Shofty et al., 2019). White matter is found in the subcortical of the brain. It contains nerve fibers called axons, which are connecting the nerve cells called neurons as shown in Figure 2.1. It consists of myelinated axons that connects different parts of grey matter. The axons of white matter are heavily covered by myelin (Song et al., 2019). The myelin gives white matter its white colour since the white matter has high concentration of myelin. Addition, the axons are in parallel arrangement called nerve fibre bundles.

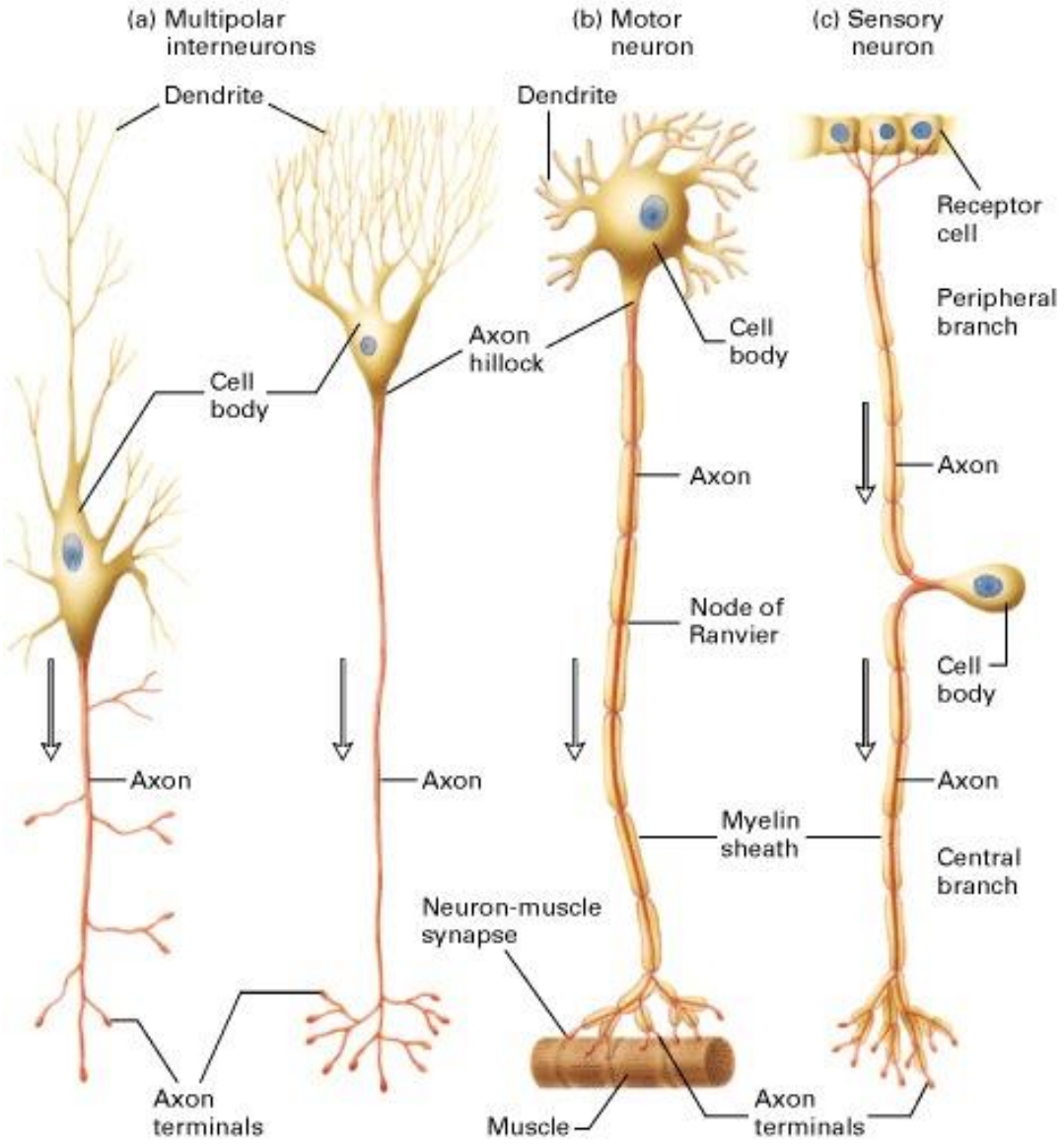

Figure 2.1 Structure of neural cell (Molecular Cell Biology. 4th edition. New York: W. H. Freeman, 2000)

2.2 Principles of diffusion magnetic resonance imaging

Diffusion magnetic resonance imaging uses pulsed gradient spin echo sequence (PGSE) which is introduced by Stejskal and Tanner in 1965. Based on the Stejskal-Tanner sequence, 90° and 180° RF pulses are applied and a symmetric diffusion sensitizing gradient pulse inserted before and after 180° refocusing pulse as shown in Figure 2.2. The gradients inserted using precisely controlled duration and distance. The proton spins dephased by the first gradient pulse and rephased by the

second gradient pulse when there are immobile water molecules between the applications of the two gradients. However, the proton spins will not be rephased by the second gradient if there are water molecules move in the direction of the gradients during the interval between the two gradients are applied. This means they dephase with regard to the hydrogen nuclei of the immobile water molecules. Hence, the faster the water molecules diffuse, the more dephased of proton spins. Thus, the echo will have a weakened signal (de Figueiredo et al. 2011, Mukherjee et al. 2008).

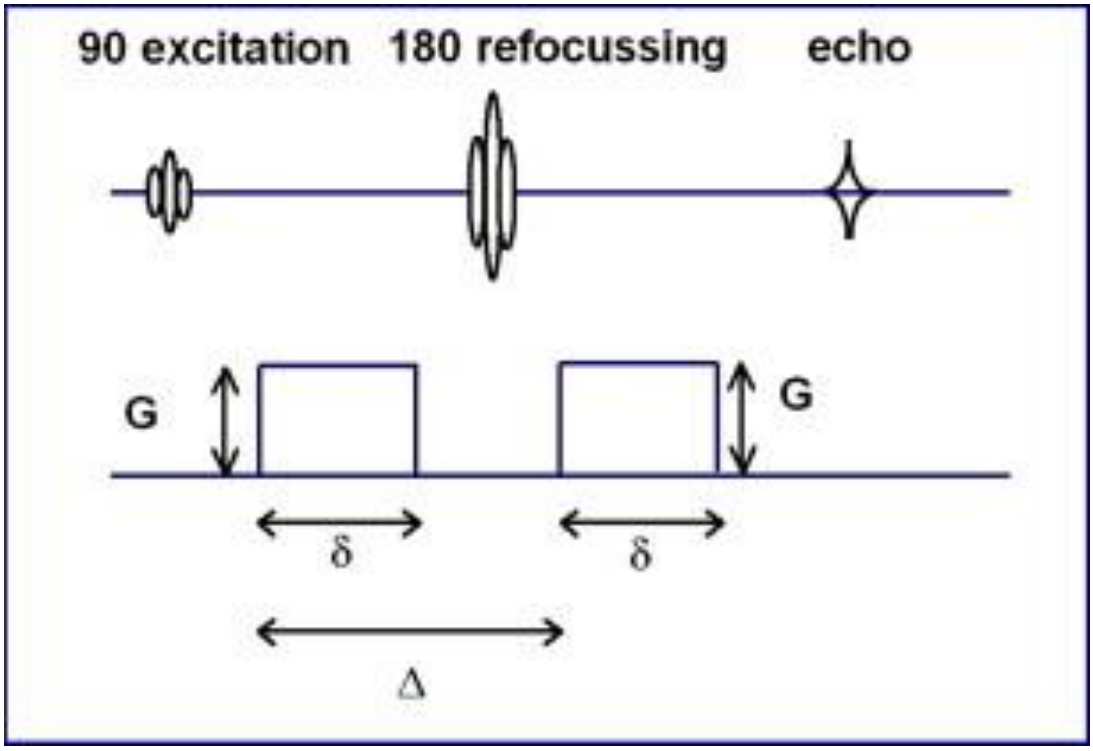

Figure 2.2 Schematic diagram of the pulsed gradient spin echo sequence introduced by Stejskal and Tanner where G is amplitude, δ is duration of the sensitizing gradient and Δ is time between the two sensitizing gradient lobes (de Figueiredo et al., 2011)

MR signal intensity is generated by proton density (PD), longitudinal relaxation time (T1), transversal relaxation time (T2), physical properties of water molecules and diffusion coefficient (D) (Mori & Zhang, 2006). The signal intensity described in a diffusion image could be expressed as Eq 2.1:

$$S = PD\left(e^{-\frac{TE}{T2}}\right)(e^{-bD}) \quad 2.1$$

where S is signal intensity, PD is proton density, TE is echo time, T2 is transversal relaxation time. D is diffusion coefficient in units of mm^2/s and b is b-value in units of s/mm^2. Term of PD, TR and TE are constant terms in this equation and could be simplified as S_o as follows:

$$S = S_o e^{-bD} \quad 2.2$$

The variation of the diffusion sequence to water molecules movement can be differed by adjusting the gradient amplitude, the duration of the sensitizing gradients and the time between the gradient pair (de Figueiredo et al., 2011). The b-value is a diffusion-weighting factor and it is described as:

$$b = \gamma^2.G^2.\delta^2(\Delta - \delta/3) \quad 2.3$$

where γ is the gyromagnetic ratio that has constant value of 2.675×10^8 rad s^{-1} T^{-1}, G is the strength of the diffusion-sensitizing gradients, δ is the duration of the gradient pulse and Δ is the time interval between the start of the diffusion gradient before and after 180° pulse.

Alger stated that the diffusion effect sensitivity increases when the b-value larger. Increasing δ and Δ to obtain the larger b-value factor (Alger, 2012).

2.3 Principles of diffusion tensor imaging

2.3.1 Anisotropic and isotropic diffusion

Diffusion tensor is a 3 × 3 matrix reflecting diffusion rates in different directions. The tensor can be rotated to assume a form with only 3 diagonal components which is eigenvalue. It can be visualized as a diffusion ellipsoid model in Figure 2.3.

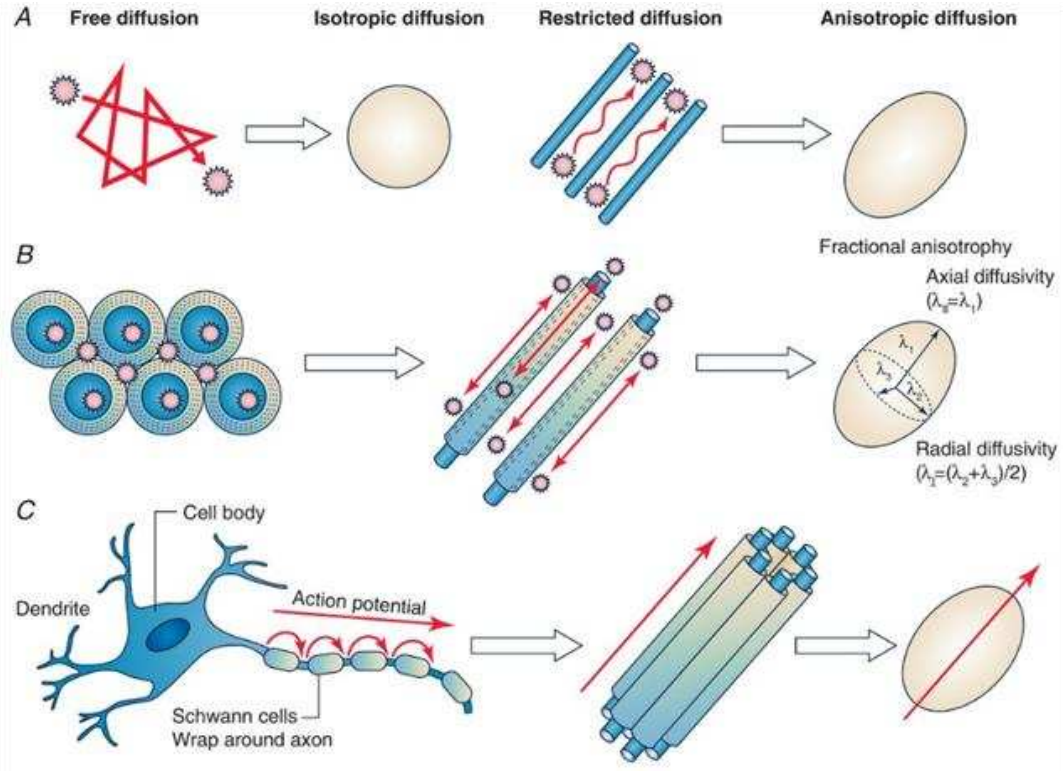

Figure 2.3 The diffusion ellipsoids model for isotropic and anisotropic diffusion (Tsai et al., 2019)

The diffusion tensor 3 × 3 matrix characterizes anisotropic diffusion where the diffusion varies with direction. Anisotropic diffusion is defined as directional of water molecules movement at a specific rate within a region (de Figueiredo et al., 2011; Salat, 2014). Biological tissue which is highly structured such as corpus callosum has a high degree of anisotropy since it is composed of tightly packed fibers. The fiber

organization makes restricted diffusion of water molecules along the fiber membrane. White matter also demonstrates high anisotropy due to parallel orientation of its nerve fiber tracts. Therefore, diffusion-sensitizing must be applied for more than one direction in order to enable the possibility of anisotropic diffusion determination.

Basser at al. (1994) show the tensor model to characterize anisotropy diffusion which is 3D Gaussian diffusion is defined as:

$$D = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \quad 2.4$$

where D is diffusion tensor while x, y and z are the three orthogonal axes.

Conversely, isotropic diffusion is characterized by single diffusion coefficient, D in which water diffusion is same in every direction. Therefore, the mobility of water molecules in random and incoherent motion in any directions. For instance, CSF spaces of the human brain has high degree of isotropy due to its microarchitecture. (Mukherjee et al., 2008).

Output of diagonalization of Eq. 2.4:

$$D' = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} \quad 2.5$$

where D' is a new tensor, λ_1, λ_2 and λ_3 are termed as eigenvalues, while v_1, v_2 and v_3 are eigenvectors that correspond to each λ_1, λ_2 and λ_3, respectively.

The eigenvalues are the values corresponding to the apparent diffusion coefficient (ADC) of the water molecules in each voxel along the direction of the eigenvectors. The eigenvectors represent the diffusivity along the principal direction and two orthogonal ones per each image voxel (Wheeler-Kingshott et al. 2012).

2.3.2 DTI Scalars

The simplest scalar derivation from DTI is the average of the three eigenvalues λ_1, λ_2 and λ_3 which called as mean diffusivity (MD) (Le Bihan et al., 2012). MD measures the average magnitude of molecular displacement by diffusion for the tensor while the sum of the tensor's eigenvalues is represented as Trace (D) (Basser et al., 2002). Meanwhile, O'Donnel & Westin (2012) contended that this average referred to apparent diffusion coeffiecient (ADC) map. Both matrices describe to the total amount of diffusion within a voxel which it is related to the amount of water molecules. Trace (D) and mean diffusivity (MD) are described by Eq. 2.6 and 2.7:

$$Trace\ (D)\ = \lambda_1\ + \lambda_2\ + \lambda_3 \qquad 2.6$$

$$MD\ = \frac{\text{Trace (D)}}{3} \qquad 2.7$$

Besides MD, other parameters that reflects the directionality of water molecular displacement by diffusion within a voxel is fractional anisotropy (FA) which is characterized by Eq. 2.8:

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}}{\sqrt{({\lambda_1}^2 + {\lambda_2}^2 + {\lambda_3}^2)}} \quad 2.8$$

Parameter FA measures the fraction of the diffusion that is anisotropic which it has range from 0 to 1. It is a normalized variance of the eigenvalues that the most widely used anisotropy measure. This parameter shows the amount of tissue organization and location that contain single white matter tract within voxel. Thus, FA could be considered as a parameter of degree of white matter (O'Donnel & Westin, 2012). Also, FA able to form color maps that it shows the direction of the water diffusion (Pajevic & Pierpaoli, 2000). The colorized image is commonly depicted in Red-Green-Blue (RGB) color system which represents the main directions of water movement in a voxel. Red, blue, and green indicate right-left (R-L), superior-inferior (S-I), and anterior-posterior (A-P) respectively.

Other relevant DTI parameters derived also axial diffusivity (AD) and radial diffusivity (RD). AD is the longest eigenvector which represents the rate of water diffusion in parallel to the white matter tract (Chanraud et al. 2010). It is described by λ_1. They also stated that RD is the average of two shorter eigenvectors which characterized by Eq. 2.9. It represents the diffusion in perpendicular to that white matter tract in voxel. Both parameters are specific markers for assessing the condition of axon and myelin of the neuronal fibres (Aung et al., 2013).

$$\lambda_1 = \frac{\lambda_2 + \lambda_3}{2} \quad 2.9$$

The mostly used scalar indices for general assessment of white matter degree are MD and FA (Rokem et al., 2017; Soares et al., 2013).

2.3.3 Fiber tractography

Fiber tractography is a method to estimate the location of white matter tracts integrated in the navigation system (Ciccarelli et al., 2008). It is a promising tool for assessing the brain white matter. Mostly the fibre tractography was based on diffusion tensor imaging in clinical practice. The local diffusion characteristic within is described by a second-order tensor for obtaining the diffusion properties within one voxel which it is calculated with the Stejskal-Tanner equation (Kuhnt et al., 2013).

A single predominant fiber orientation and pieces with those local orientations characterizing the each imaging voxel in fiber tractography (Jeurissen et al., 2017). Those local fiber orientations are recognized as a three-dimensional (3D) vector field and the global fiber trajectories as its streamlines as shown in Figure 2.4.

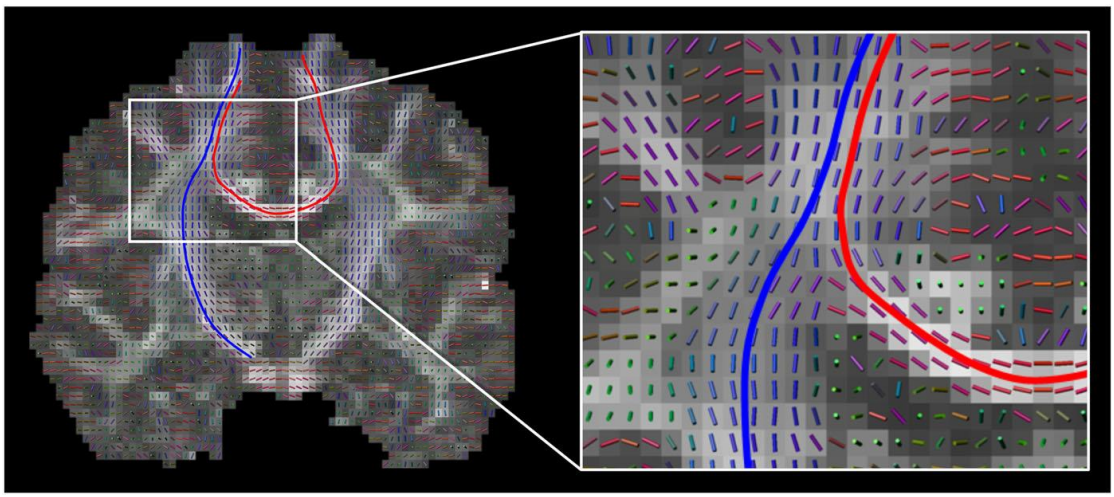

Figure 2.4 Vector field of local predominant fiber orientations and two of its streamlines depicted on a coronal view of the human brain. The blue streamline is part of the corticospinal tract, whereas the red one is part of the corpus callosum. (Jeurissen et al., 2017)

2.4 Clinical significance of DTI in brain imaging

DTI is presently a promising tool for exploring white matter architecture in brain non-invasively since it is sensitive to microstructural tissue properties. This sensitivity has made DTI widely used as a clinical tool especially white matter abnormalities (Mori & Zhang, 2006). The DTI indices such as AD, RD, MD and FA provide various information to infer the diffusion rate along the axon fibre, rate of diffusion in the transverse direction in axon, molecular diffusion rate, and directional of diffusion respectively.

Since DTI scalars could be used to assess the changes in the brain which has led to tremendous interest in DTI in clinical research. This technique has been vast in examining various disease like tumor, cancer, neurological disorder and psychiatric disease. The diseases that has been applied in DTI such as schizophrenia (Arat et al., 2015, Kubicki et al., 2007), neurological disorder (Ciccarelli et al., 2008), multiple sclerosis (Cercignani et al., 2001; Filippi & Agosta, 2010; Inglese & Bester, 2010; Sun et al., 2007; Zipunnikov et al., 2014), stroke (Mukherjee, 2005; Radlinska et al., 2010), traumatic brain injury (Maller et al., 2010), Parkinson's disease (C. Atkinson-Clement, 2017), Alzheimer's disease (Morikawa et al., 2010; Nakata et al., 2008), brain tumor (Dubey et al., 2018; Zhang et al., 2019), as well as breast cancer (Luo et al., 2019).

Furthermore, DTI technique is also implemented in study of assessment of white matter abnormalities particularly leukoaraiosis (Mohd Taib et al., 2014; O'Sullivan et al., 2004; Zhao et al., 2019) which is the major topic to be reviewed in this study.

2.4.1 Leukoaraiosis

Leukoaraiosis (LA) is a pathological appearance of white matter in the brain which appearing as hyperintensity white matter on T2-weighted image MRI brain scans or CT scans. As can be seen in Figure 2.5, the term "leukoaraiosis" originally from Greek which "leuko" referred to white and "araiosis" referred to rarefaction (O'Sllivan, 2008). It was first described by Hachinski (1987) over 30 years ago regarding its pathological and introduced as an imaging diagnostic terminology. Generally, the terms were often used synonymously to describe LA such as white matter lesion (WML), white matter hyperintensity (WMH) and white matter changes (WMC). All these terms are categorized as cerebral small vessel disease (CSVD) as referring to the presence of white matter spots in the brain (Wardlaw et al., 2013). Other a few terms were also used in previous studies is white matter disease (WMD), white matter damage and ischaemic white matter disease.

LA is often seen in the elderly and white matter changes related with increased age. It is also commonly related with vascular risk factors such as hypertension, cognitive decline (Zhao et al., 2019) and diabetes mellitus (Zupan, 2016). Smith (2010) revealed that LA may be a marker of stroke since it is a commonly seen in stroke patients. Also, it was also observed in patients diagnosed with Alzheimer's disease (Scheltens et al., 1992) and many other diseases such heart diseases, and type 2 diabetes.

Furthermore, the neuropathological studies revealed that LA associated to demyelination, gliosis, axonal loss and perivascular spaces (Zupan, 2016). Some studies have found a relationship between leukoaraiosis and depression.

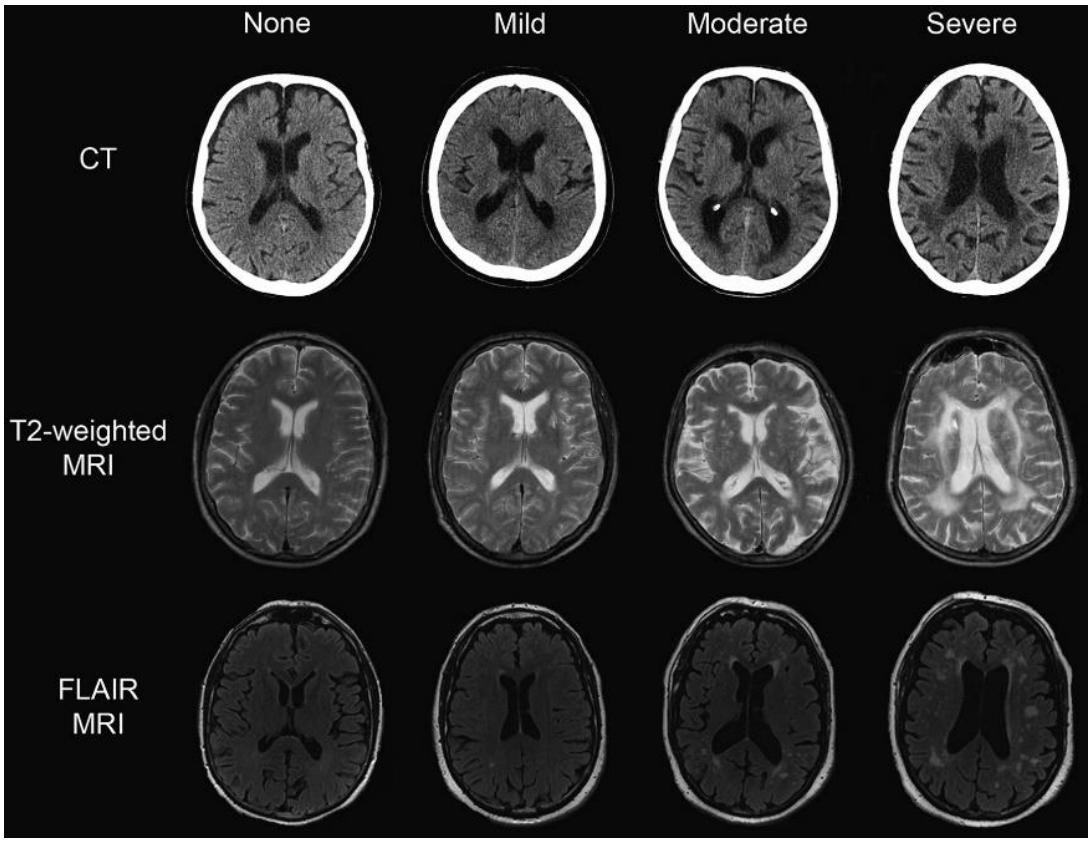

Figure 2.5 Stages of leukoaraiosis severity showed in CT scan and T2-weighted MRI (Grueter & Schulz, 2012)

CHAPTER 3

GENERAL METHODOLOGY

3.1 Data acquisition

This is a retrospective study based on data images obtained from longitudinal study on Tocotrienol (2008-2012) performed in collaboration by AMDI, School of Pharmaceutical Sciences and School of Computer Science (Goplan et al., 2014).

Data images of 43 subjects were retrieved from USM PACS system and sent to an independent workstation for post-processing and further analysis. The data consist of DTI and FLAIR images obtained using 1.5 Tesla MRI System (Signa HDxt, GE Healthcare available at AMDI-USM. This study was approved by Human Research Ethics Committee USM (USM/JEPeM/17050265).

It was reported from previous study that all subjects participated in the study were of those who have no any neurologic illness. Subjects were interviewed and underwent health screening to ensure that all subjects involved in the study are healthy and that the lesions developed in the subject's brain are truly due to aging process (Mohd Taib, 2014).

It was reported that all data were acquired using the protocols as listed in Table 3.1. (Mohd Taib et al., 2017).

Table 3.1 DTI protocols applied during data acquisition

Protocols	Value
TR	17000 ms
TE	101.1 ms
b-value	0 and 1000 s/mm^2
No. of diffusion encoding directions	30 with 3 sets of unweighted (b_o) images, 62 axial slices parallel to AC – PC line
Slice thickness	2.0 mm with no interslice gap
Matrix size	128 × 128 (zero-filled and reconstructed to 256 × 256
FOV	26 cm × 26 cm
Isotropic voxel resolution	2.0 mm

Sample size was calculated using sample size calculator software (https://wnarifin.github.io/ssc_web.html), based on comparison of two mean for power of study of 80 % and confidence interval of 95 %. 41 samples data were needed for this study. However, the available images is 43. Therefore, all 43 images were analyzed. It was reported that subjects were recruited based on the following inclusion and exclusion criteria.

3.1.1 Inclusion criteria

3.1.1 (a) All subjects that have been scanned for previous study (Mohd Taib, 2014)

3.1.1 (b) The age of subject is 18 years old and above.

3.1.2 Exclusion criteria

3.1.2 (a) Incomplete images

3.1.2 (b) Images with artefacts

3.2 Data analysis

Measurement of leukoaraiosis volume and DTI values, specifically MD, AD RD and FA was performed using Osirix MD version 4.1.2 (Rosset et al., 2004). Specific method of analysis are described in Chapters 4 to 8 which represent different types of analysis. Each type of analysis correspond to specific objectives of this study, as mentioned in section 1.4.2 in this thesis.

3.3 Placement of ROI

Basically, all ROIs drawn were drawn on the MD maps, which was then copied and pasted on the AD, RD, and FA maps to ensure an accurate and standardized placement of the ROIs on all parametric map. Then, the values displayed on each map were recorded.

However, detail description on placement of ROI are described in Chapters 5 to 8 which correspond to different types of analysis as outlined in the objective of the study.

CHAPTER 4

DEMOGRAPHIC ANALYSIS

4.1 Introduction

Demographic analysis was performed to describe the distribution of gender, age, number of lesion spots and lesion size of all subjects that participated in this study.

4.2 Method of analysis

Descriptive analysis was performed in which subjects were divided into four categories according to their genders, age group, number of lesions group and lesion volume sizes group. The first category is number of lesions based on the gender. The second category is classification of lesion sizes based on the gender. The third category is number of lesions based on the age. The fourth category is classification of lesion sizes based on the age.

Independent T-test was performed to compare means in order to determine the relationship between gender and number of lesions. For all comparisons, $p < 0.05$ was considered statistically significant.

4.3 Results and discussion

The number of subjects that participated in this study was 43 in total. There are 17 male subjects (39.5%) and 26 female subjects (60.5%). Their ages were in range from 39 years old to 67 years old (Mean = 54.47, SD = 7.229). The number of lesion spots detected in brain white matter for all subjects was in range from 1 to 19 spots (Mean = 6.37, SD = 4.408).

The highest age group of participated subjects was in range from 50 to 59 years old. However, for the youngest subject was 39 years old and the only one subject that comes from the range of 30 to 39 years old.

All those demographic data are summarized in Table 4.1.

Table 4.1 Demographic analysis of 43 subjects

Demographic profile		Number of subjects
Gender	a) Male	17
	b) Female	26
Age group	a) 30 – 39 years old	1
	b) 40 – 49 years old	11
	c) 50 – 59 years old	18
	d) 60 – 69 years old	13

4.3.1 Gender and number of lesions

The number of lesions for male was in range of 1 to 19 lesion spots (Mean = 6.65, SD = 5.219), whereas for female was in range of 1 to 15 lesion spots only (Mean = 6.19, SD = 3.889).

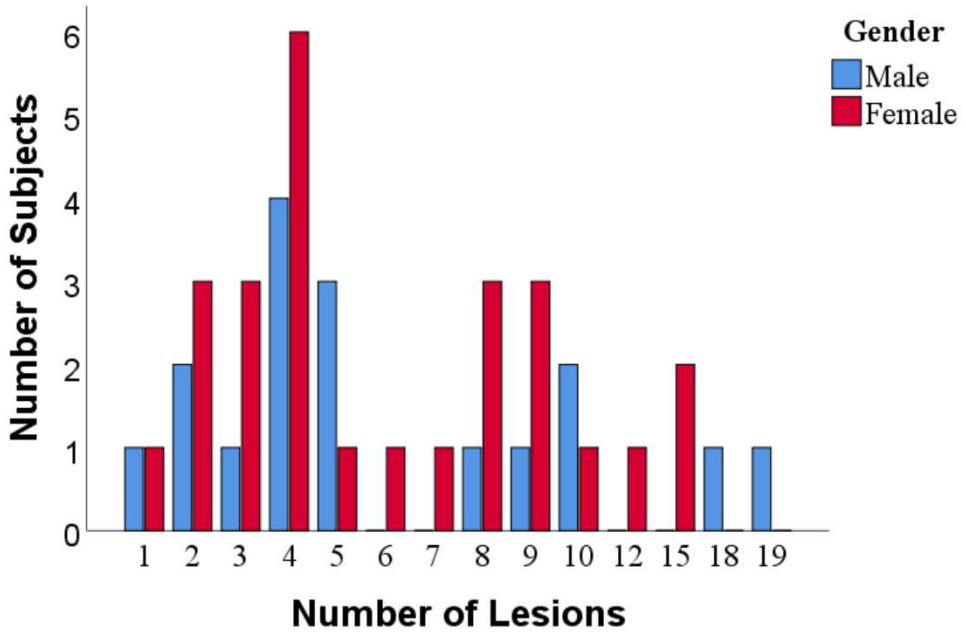

Figure 4.1 Bar chart showing the number of lesions based on gender.

In Figure 4.1, for both groups of male and female subjects, most subjects had 1 to 10 lesion spots compared to subjects with a number of lesion spots above 10. The number of lesion spots of 1 to 9 are more in female subjects because the number of female subjects themselves is more than male subjects.

In addition, independent T-test was used to determine the relationship between gender and number of lesions. Based on the independent T-test result, *p*-value = 0.62. Thus, there was no significant difference of mean number of lesions between male and female.

A previous research did not find any gender differences in the prevalence of leukoaraiosis (Grueter and Schulz, 2011). However, Van Dijk et al., (2008) and van den Heuval et al. (2004) revealed that the female has higher prevalence of leukoaraiosis. Gender differences may affect the pathogenesis of white matter lesions.

4.3.2 Gender and lesion sizes

The lesions was classified into four groups depends on their sizes. Those are small (S<1.0 cm^3), medium (1.0≤*M*<5.0 cm^3), large (5.0 cm^3≤*L*<10.0 cm^3) and very large (*VL*≥10.0 cm^3). The categorization of these lesion sizes is based on the previous study (Mohd Taib et al. 2014).

Based on Figure 4.2, the majority for both male and female subjects have small lesions. Only two subjects (1 male and 1 female) had medium size lesions. Only one subject (male) had a large lesion.

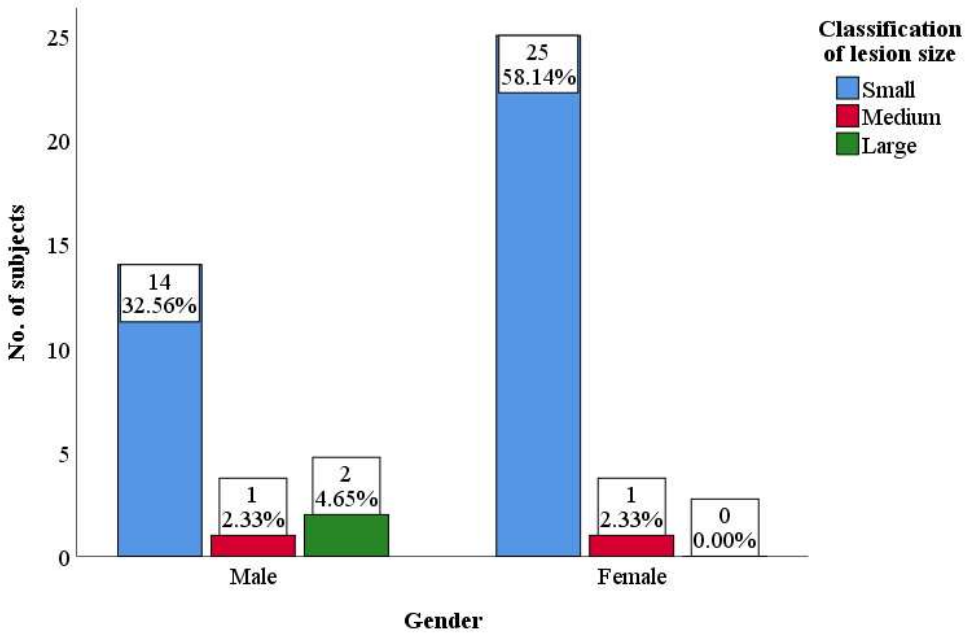

Figure 4.2 Bar chart showing the classification of lesion size based on the gender.

In this finding, it was found that women have higher small lesion sizes. Women have a higher prevalence and severity of leukoaraiosis than men reported in some studies (de Leeuw et al., 2001). Van Dijk et al., (2008) reported that higher progression