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Diffusion tensor imaging (DTI) studies of cerebral white matter integrity in normal to moderate cardiovascular risk patients

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Abstract

Background & Objectives: The cerebral small vessel disease (CSVD) manifest as white matter lesion (WML) in magnetic resonance imaging (MRI). The diffusion tensor imaging (DTI) study shows a lower white matter integrity in white matter lesion (WML) and its penumbra region, suggesting cerebral white matter damage. The aim of this study is to establish correlation between the DTI values of white matter with the age related white matter changes (ARWMC) visual rating score and identify predictors of ARWMC score. *Methods*: 63 brain MRI images of subjects were selected. Relevant clinical information of the subjects which satisfied QRISK2 risk factors were retrieved from the patients' folder. DTI parameters were obtained via postprocessing at OsiriX DTImap workstation. *Results*: FA frontal (r = -0.36; $p = 0.003$) and AD frontal (r = -0.26; $p = 0.040$) had a substantial and negative correlation with the ARWMC score. There was a significant positive correlation shown between the ARWMC score and RD frontal (r = 0.30; $p = 0.018$). There were positive significant association between the ARWMC score and age (b(95%CI): 0.106 (0.061, 0.151); p<0.001) and QRISK2 score (b(95%CI): 0.235 (0.036, 0.433)). *Conclusion*: DTI is a good method to study the cerebral white matter integrity. It was found that frontal lobe is the first region affected in relation to ageing, in particular the myelin integrity. Increasing age and higher QRISK2 cardiovascular risk factors were shown to increase the ARWMC score.

Keywords: DTI studies, age related white matter changes, visual rating score, cerebral white matter, normal appearing white matter

INTRODUCTION

Stroke is a global health problem due to it being the second most common cause of death and the world's leading cause of adult disability. Annually 15 million people worldwide suffer from stroke. Out of these, about one third die and another third are left permanently disabled. Malaysian Ministry of Health statistics has consistently shown stroke as one of the top five leading causes of death since the year 2000. Data from 2009 showed cerebrovascular disease causing a mortality of 8.43 per 100,000 population.[1]

Atherothromboembolism, intracranial small vessel disease (penetrating artery disease) and cardiogenic embolism are the three main causes of ischaemic stroke. Of these, cerebral small vessel disease (CSVD) accounts for one fourth of stroke cases.[1] In magnetic resonance imaging (MRI), the markers for CSVD are white matter lesion (WML) and lacunar infarct. WML is defined as high signal intensity changes of the cerebral white matter in T2 weighted or fluid attenuated inversion recovery (FLAIR) sequences.[2]

Multiple visual rating scores are available to quantify WML, namely Fazekas, Wahlund and Scheltens scoring. The scores are based on numbers and determined by the identification of lesions. The vascular cognitive impairment harmonization standard recommends the age-related white matter changes (ARWMC) scale as the preferred visual rating scale. This scale can be applied to both MRI and CT with moderate to excellent reliability.[3] In the ARWMC scale, the degree of white matter changes is rated on a 4-point scale. Changes in the basal ganglia are rated in the same way and considered as WML even if located in the grey matter nuclei, which contain a small amount of white matter.[2]

Cardiovascular risk factors are shown to have a relationship with the cerebrovascular disease. Both

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macrovascular diseases such as hypertension and atherosclerosis, and microvascular diseases such as endothelial dysfunction have been associated with CSVD.[4] Age, hypertension (diastolic blood pressure), hyperlipidaemia, diabetes mellitus and smoking duration were strongly associated with WML in some studies[5,6] in a South Asian population, more so than in a European population.[6] There are few known multivariable risk scores used to project patients' risk of suffering from cardiovascular disease within 10 years after taking the test. The QRISK2 score used in this study is an improved version of the QRISK1 which include additional risk factors such as self-assigned ethnicity and conditions associated with cardiovascular risk.[7] The risks are stratified into high risk (> 20%), intermediate risk (10%–20%), and low risk (< 10%).[8]

Diffusion tensor imaging (DTI) is a relatively recent quantitative MRI technique. It allows an in vivo study of tissue microstructure which is used to study cerebral white matter. DTI provides multiple imaging metrics, such as fractional anisotropy (FA) and mean diffusivity (MD). These metrics have been shown to detect changes in white matter microstructure in which conventional MRI are not able to.[9] It measures how water moves within the tissue microstructure. Measurement is derived through the application of a magnetic diffusion gradient in the different areas (at least 6) to get a diffusion tensor.[10] A lower FA and a higher MD reading would likely indicate a lower microstructural connectivity.[11] There are other tensor indices that are used to determine neuronal damage, namely axial diffusivity (AD) and radial diffusivity (RD). AD reveals the axonal status and the RD assess the myelin changes.[12] There are 3 methods used for the post processing procedures. They are region of interest (ROI), Tract-based spatial statistics (TBSS), and voxel-based analysis. The ROI technique seems to be the best method because the measurement is more uniformed across the different subjects and increases the reliability of the data captured by focusing on the relevant regions.[10]

Literature shows that advanced age has been associated to vascular risk factors, cerebral hypoperfusion or compromised blood brain barrier integrity.[13] A study revealead a positive correlation between the total WML load and diffuse WM injury in normal appearing white matter (NAWM), suggesting cerebral white matter damage to be more widespread rather than region specific. Normal white matter, WML penumbra, and WML all show a similar decline in WM integrity over time.[14] Accordingly, NAWM regions that progress into WML already had a significantly lower FA and higher MD at baseline imaging compared with persistent NAWM.[10] Consequently, the information gathered enable the researchers to study the NAWM integrity in the different regions of the brain and to predict the presence of WML development and WM injury.[15] In summary, DTI in MRI studies enables the investigation of normal appearing white matter (NAWM) microstructures before WML develop.[16]

Therefore, the purpose of this study was to evaluate the microstructure of the cerebral white matter using DTI. All four DTI parameters namely FA, MD, RD and AD were measured and correlated with the ARWMC visual rating score. The significant predictors to the ARWMC score were identified.

METHODS

Data collection

This was a cross sectional study conducted at the Department of Radiology, Hospital Universiti Sains Malaysia (HUSM), Kelantan, Malaysia. This study has been approved by the Human Research Ethics Committee of our university (JEPeM code: USM/JEPeM/15030096). A total of 63 brain MRI images of subjects from Kelantan who attended the Klinik Rawatan Keluarga (KRK) Department, HUSM which fulfilled the QRISK2 risk factors were retrieved from the patients' folders. The completed images were retrieved from the PACS at the Department of Radiology, HUSM. Incomplete study, study with degraded image quality and non-availability of the study within PACS were excluded from the study. Apart from that, patients with previous history of cerebrovascular disease, neurological disorder, brain tumour, congenital brain anomaly and high cardiovascular risk factors were excluded earlier by the principal investigator.

All MRI examinations were performed using the same Philips 3-Tesla Achieva MR scanner (Best, The Netherlands). All collected MR-DTI output from the study subjects were manually analysed. Post processing and analysis were carried out using OsiriX software version 4.1.2 (17). The MRI examinations were labelled with serial numbers in order to maintain the privacy and confidentiality of the subjects.

The MR protocol included T1 axial (TE / TR 10/600 ms, matrix 512 x 512; FOV 230.0x 230.0, NEX 1.0; slice spacing 1.0mm, slice

thickness 5.0mm, flip angle 70), 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), 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), DTI 32 (TE/TR 72/10000ms, matrix 96X 96; FOV 240.0x 240.0, NEX 1.0; slice spacing zero, slice thickness 2.5mm, flip angle 90; b value 0).

Neuroimaging assessment

Images were reviewed in the PACS UV workstation. The qualitative measurement assessed the WML in the brain MRI images. The subjects were further categorised using the age related white matter changes (ARWMC) score (Table 1). FLAIR and T2 weighted axial images were reviewed to determine the scoring. The following brain areas were used for rating: frontal, parieto-occipital, temporal, infratentorial, and basal ganglia (globus pallidus, thalamus, internal/external capsule, and insula). The rating of these five regions were done in the right and left cerebral hemispheres separately. Total score ranged from 0-30.

Image processing and quantitative measurement

Post processing and analysis was carried out using OsiriX software version 4.1.2.[18] Diffusion tensor elements and anisotropy at each voxel were computed. FA, AD, RD, MD and colour coded FA were constructed. Regions of interest (ROIs) were placed on the FLAIR images. Small oval ROIs of $0.30 \pm 0.02 cm^2$ were drawn manually (Figure 1).[17] The ROIs were placed in five different regions and rated in the right, left hemispheres, basal ganglia and brainstem separately as stated below[2]:

ROIs were sampled on 5 contiguous sections for the right and left frontal, temporal and parietal lobe white matter area.[19] The ROIs were put approximately 2mm from the frontal horn of both lateral ventricles, at each section. In the temporal lobe, ROIs were put approximately 2mm posterolateral to the temporal horn of the lateral ventricle. As for the parietal lobe, ROIs were placed approximately posterior to the central sulcus on the most superior section in which the centrum semiovale is clearly visible and the subsequent four sections were 2mm from the body of the lateral ventricle. The ROIs in the occipital lobe were placed on 3 contiguous sections, 2mm from the occipital horn of the lateral ventricle.

The ROIs for the basal ganglia, which included the globus pallidus, thalamus, internal, external capsules, and insula were drawn in a single axial slice, the largest size for that structure.[20] The ROIs for the infratentorial area which included the brainstem (pons and midbrain) were placed in 3 contiguous sections at right and left, the largest size for the structures. The means of right and left white matter/basal ganglia/infratentorial (FA, MD, AD, RD) were taken separately and averaged before undergoing analysis.

Statistical Analysis

IBM SPSS Statistics software (version 24) was used for the data analysis. All data were cleaned and explored. Numerical variables were presented using mean and standard deviation after normality assumption checking, while categorical variables were presented using frequency and percentage (Table 2). Average DTI parameters were calculated with readings from both sides (left and right side).

Spearman's correlation was used to check the correlation between the DTI parameters and ARWMC score, and multiple linear regression was carried out to test the association between the

Table 1: ARWMC visual rating score

ARWMC rating scale for MRI White matter lesions	
0	No lesions
1	Focal lesion
2	Beginning confluence of lesions
3	Diffuse involvement of entire region, with or without involvement of U fibres.
Basal Ganglia lesions	
0	No lesion
1	1 focal lesion >5mm
2	More than 1 focal lesion
3	Confluent lesion.

ARWMC: Age related white matter changes

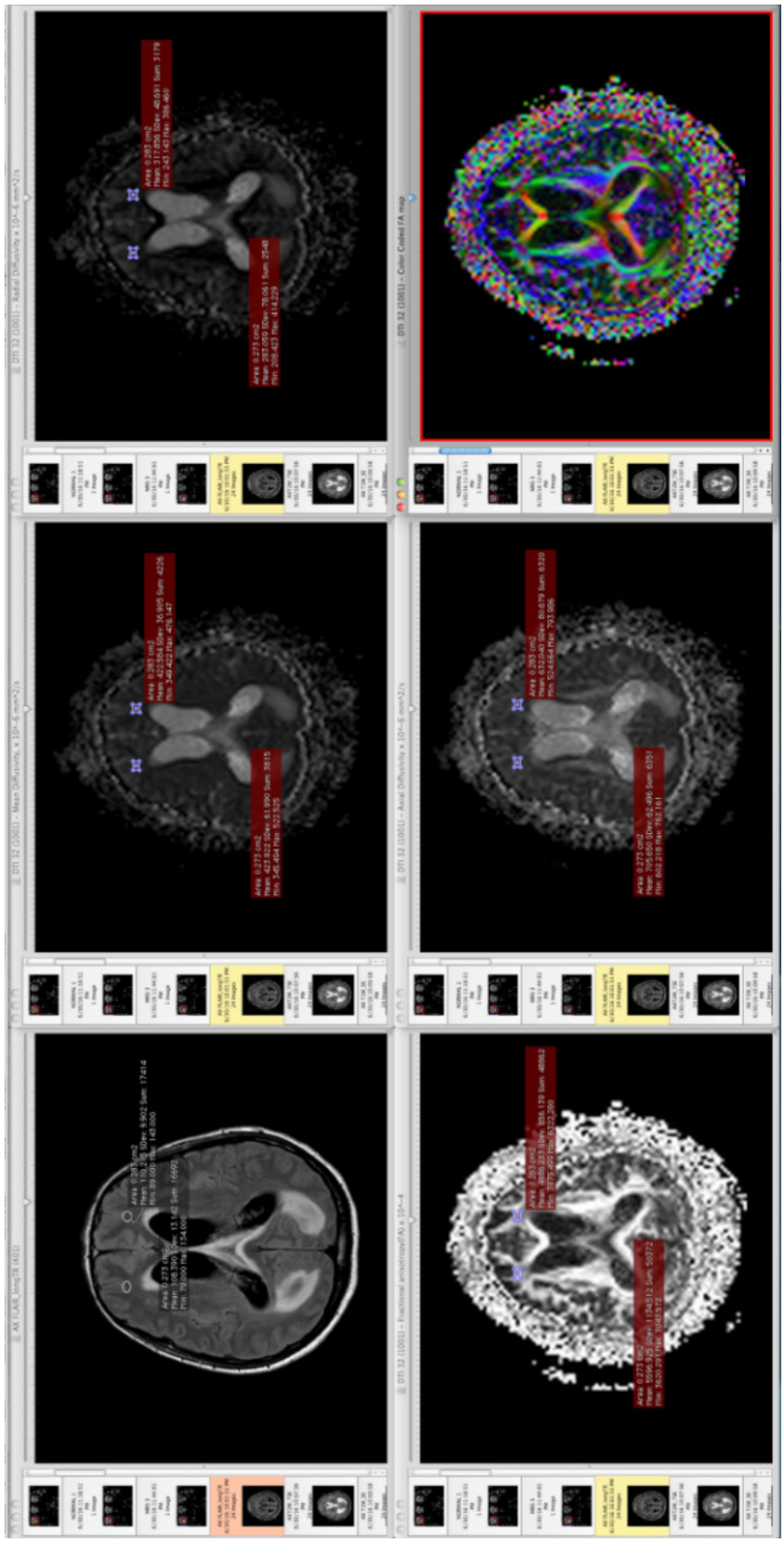

Figure 1. ROI placement at specific cerebral region (OsiriX). The ROI size 0.30 ± 0.02 cm^2 is manually drawn on the FLAIR images at the selected region (in frontal, temporal, parietal, occipital, internal capsule, thalamus, external capsule, infratentorium and insular) and simultaneously coordinated on FA, MD, AD and RD maps.

Table 2: Characteristic of study population (N = 63)

Variables	Mean (SD)	Range	Frequency (%)
Age	40.10 (11.45)	25.00 – 62.00	
Gender			
Female			43 (68.3)
Male			20 (31.7)
Race			
Chinese			5 (7.9)
Malay			56 (88.9)
Others			2 (3.2)
Comorbidities			
No			41 (65.1)
Yes			22 (34.9)
ARWMC	1.79 (2.40)	0 – 9.00	

SD: Standard Deviation

DTI parameters and ARWMC score. For multiple linear regression, interaction and multicollinearity checking were done. Model assumptions were checked as well to indicate the model fitness. A p value < 0.05 was considered statistically significant.

RESULTS

A total of 63 patients completed the brain MRI as per protocol. The mean age of participants was 40 years old (range 25–62 years old). The mean ARWMC score was 1.79 (range 0–9). Two thirds of the patients were female (43 patients were female, 20 were male) and one third of the patients had associated comorbidities (hypertension, hyperlipidaemia) (Table 1).

Thirty one patients were categorised as normal (no cardiovascular risk factors) while 27 and 5 patients were within low risk and moderate risk respectively (Table 2). Of these, 23 patients have comorbidities (37%) (Table 3).

The DTI parameters of FA, MD, AD & RD were presented with their means and standard deviations in Table 4. All data were normally distributed. Outlier results due to errors in data entry were not observed.

Table 5. summarises the relationship of the DTI parameters and ARWMC score using Spearman's correlation as the distribution of the ARWMC score was positively skewed. Overall, the present study observed that the DTI parameter FA frontal (r = -0.36; p = 0.003) and AD frontal (r = -0.26; p = 0.040) had substantial and negative correlation with the ARWMC score. In addition, there is a significant positive correlation shown between the ARWMC score and RD frontal (r = 0.30; p = 0.018).

Table 6. reveals the association between different DTI parameters and the ARWMC score with the variable selection stepwise method used. The results revealed significant association between the ARWMC score and age, RD insular and QRISK2 cardiovascular risk factors. There were positive significant association between the ARWMC score and age (b(95%CI): 0.106 (0.061, 0.151); p < 0.001). This indicates that a one year increase in age would result in an increase of the ARWMC score by 0.106. There was also positive significant association between the ARWMC score and QRISK2 cardiovascular risk factors (b(95%CI): 0.235 (0.036, 0.433); p < 0.021) which indicates that increased cardiovascular risk factors would result in an increase of the ARWMC score by 0.235.

Table 3: Cardiovascular risk factors patients, QRISK2 (N = 63)

QRISK2	Frequency (%)	ARWMC Score	Comorbidities
Normal	31(49%)	0,1,2,3,5,6	9(29%)
Low risk	27(42.8%)	0,1,2,4,5,7,9	11(40.7%)
Moderate risk	5(8%)	3,6,8,9	3(60%)

Table 4: DTI parameters of study population (N = 63)

Variables	FA Mean (SD)[a]	MD Mean (SD)[a]	AD Mean (SD)[a]	RD Mean (SD)[a]
Frontal	6497.31 (500.27)	399.30 (17.75)	739.34 (48.54)	229.80 (25.57)
Temporal	6997.17 (493.46)	402.46 (18.97)	782.92 (41.30)	214.48 (28.94)
Parietal	7275.20 (503.83)	377.04 (15.96)	775.18 (48.37)	178.55 (25.88)
Occipital	7730.45 (530.91)	389.58 (17.54)	847.34 (59.30)	160.62 (32.11)
Infratentorium	7616.89 (474.49)	382.24 (22.28)	806.17 (69.93)	170.23 (25.10)
External capsule	6069.67 (615.26)	394.84 (22.04)	711.59 (70.87)	237.77 (50.94)
Internal capsule	8777.08 (428.39)	367.74 (19.29)	934.91 (57.10)	85.48 (25.84)
Lentiform nucleus	3007.06 (495.78)	374.17 (19.95)	493.42 (35.20)	321.38 (46.46)
Thalamus	5486.40 (668.07)	360.37 (22.90)	590.26 (41.34)	247.06 (31.46)
Insular	3054.83 (651.55)	523.67 (73.80)	688.13 (79.57)	435.51 (81.19)

SD = Standard deviation; FA = Fractional anisotropy; MD = Mean diffusivity; AD = Axial diffusivity; RD = Radial diffusivity

[a] FA values are X 10^{-4}, MD, RD and AD values are mm^2s^{-1} X 10^{-5}

In contrast, there was a significant negative association between the ARWMC score and RD insular (b(95%CI): -0.007 (-0.013, -0.001); p = 0.031). This indicates that a unit increment in RD insular would result in a decrease in the ARWMC score by 0.007 on average. The model explained 30.6% of variance in the dependent variable ARWMC (R^2 = 0.298). There was no interaction between age and RD insular.

DISCUSSION

This study aimed to provide a quantitative assessment of the white matter integrity in adults

Table 5: Correlation of DTI parameters and ARWMC score (N = 63)

Variables	FA r (*p*-value[1])	MD r (*p*-value[1])	AD r (*p*-value[1])	RD r (*p*-value[1])
Frontal	-0.363 (0.003*)	-0.035 (0.785)	-0.259 (0.040*)	0.298 (0.018*)
Temporal	0.093 (0.468)	-0.103 (0.423)	0.165 (0.198)	-0.238 (0.061)
Parietal	0.026 (0.839)	-0.003 (0.982)	-0.103 (0.424)	-0.004 (0.973)
Occipital	-0.086 (0.504)	0.107 (0.405)	-0.018 (0.886)	0.123 (0.337)
Infratentorium	0.045 (0.726)	-0.046 (0.721)	-0.026 (0.839)	-0.075 (0.562)
External capsule	0.230 (0.070)	-0.199 (0.118)	0.081 (0.527)	-0.222 (0.080)
Internal capsule	-0.014 (0.915)	0.000 (0.998)	-0.012 (0.926)	0.050 (0.696)
Lentiform nucleus	0.211 (0.096)	0.022 (0.863)	0.092 (0.472)	-0.062 (0.632)
Thalamus	0.060 (0.640)	0.003 (0.981)	-0.047 (0.717)	0.016 (0.899)
Insular	0.007 (0.955)	0.035 (0.788)	-0.061 (0.634)	-0.087 (0.496)

SD = Standard deviation; FA = Fractional anisotropy; MD = Mean diffusivity; AD = Axial diffusivity; RD = Radial diffusivity

*significant at 0.05 level.

[1]Spear correlation tests were applied

Table 6: Association of DTI parameters and ARWMC using multiple linear regression

Variables	Adj b (95% CI)	t-stat	*p* value
Age	0.106 (0.061, 0.151)	4.70	<0.001
RD Insular	-0.007 (-0.013, -0.001)	-2.21	0.031
Gender	-1.53 (-1.069, -1.375)	-2.51	0.803
Comorbidity	-0.634 (0.516,-1.783)	-1.104	0.274
QRISK2 cardiovascular risk factors	0.235(0.036,0.433)	2.367	0.021

Variable selection stepwise method was done, there were no multicollinearity and interaction. Model assumptions were found fulfilled

R^2 = 0.306; Std. Error = 2.04; F statistic (df) = 13.21 (2, 60)

The model is able to explain 30.6% of variance in ARWMC (R^2 = 0.306).

who have normal to moderate cardiovascular risk factors by comparing the MD, AD, RD and FA values of white matter with the ARWMC score. The investigation also included the grey matter of basal ganglia namely the thalamus, internal capsule, external capsule, insula and lentiform nucleus.

In this study, we found that the FA values of both frontal lobe white matter were significantly lower with increasing ARWMC score. A lower FA value denotes increasing WML severity.[21,22] Previous journal articles reported that WML have a significantly lower FA value with higher MD value.[23] The explanation for this finding is a reduction in the amount of myelin sheath[24], axonal loss[25] and subsequent gliosis[21] which leads to a less restricted water molecule movement in regards to its specific direction. Thus, the lower FA values in both frontal lobe white matter signify the start of white matter deterioration.[19,22]

We found that FA value in temporal, parietal and occipital lobes had no significant correlation with the ARWMC score. The possible explanation for this finding is the age-related changes that are primarily situated in the frontal lobe or caused by certain deterioration of specific white matter tracts.[22] In other studies, it was learned that the propensity for this occurring is concentrated in the frontal and parietal lobes in comparison to the occipital and temporal lobes, following the inferior-to-superior gradient rather than posterior-to-anterior gradient.[26]

Conversely, we found that there was a significant increase of frontal RD and decreased AD values with an increased ARWMC score. DTI ageing studies research showed AD reflecting axonal differences (e.g., axonal damage or loss) and RD reflecting myelin integrity.[27] An analysis of the age-related differences in AD and RD showed that higher RD value when compared to AD was interpreted as a myelin-specific effect due to ageing.[21,28,29] Previous reported DTI studies revealed a declining FA value among older adults is usually linked to age-related increase in both RD and AD.[30-32] In contrast, the present study showed that a decline of FA in patients led to a rise in RD and a drop in AD value in the frontal lobe. This pattern was recently discovered and reported in a DTI ageing study[33], where it occurred in a single white matter region in the frontal lobe specifically the anterior pericallosal, anterior/superior corona radiata.[27,34] The possible explanation for this newly found pattern is the occurrence of an ischemic incident followed by axonal degeneration and subsequent gliosis, which leads to the interruption of diffusion coherence and reduces diffusion parallel to the main diffusion direction.[33-35]

Another explanation for age-related AD decrease might be the disruption of macrostructural organisation involving multiple crossing white matter tracts leading to a less coherent diffusion and decreased AD reading, more pronounced in older adults due to reduced axonal packing contributed by the age related demyelination and axonal shrinkage.[36,37] This is not the case in young adults as the multiple crossing white matter tracts are highly aligned and packed as opposed to older adults.[38] Otherwise, there is little that we know of AD and RD because most of the studies and research papers have primarily focused on FA and MD.[39]

This study revealed that age has a positive association with the ARWMC score. An increment in age would result in an increasing ARWMC score. This is supported by most of the previous studies.[11,31] Gender and comorbidity have no significant association with the ARWMC score. We found that cardiovascular risk factors based on QRISK2 showed a positive association with the ARWMC score indicating that increased QRISK2 cardiovascular risk factors would increase the ARWMC score. These findings are compatible

with a recent study that showed that older age and higher cardiovascular risk factors are associated with an increasing WML burden in a South Asian population, as compared to a European one.[6]

We also found that the RD insula cortex showed a significant negative association with the ARWMC score. Very little study on the DTI values of insula alone has been done previously. The insula cortex contains more of grey matter, less myelin in comparison to white matter. Thus the lack of myelin will increase the RD value denoting a less restricted water diffusion.[40]

There are some limitations in this study, including the use of a linear model to examine age related tissue changes and low correlations between the DTI indices and age in certain brain areas. We found several brain sites that showed age-related tissue changes with diffusion values, indicating sufficient statistical power to examine those areas. However, the small number of subjects may have precluded showing tissue changes in other brain sites because of insufficient statistical power; some of those areas appeared in previous studies.[41] Another limitation was the ROI method to obtain the DTI values. The preselected ROI tends to be slightly varied in each subject despite a uniform size being used.

In conclusion, DTI is a powerful method for characterising changes in tissue microstructures associated with ageing. It was found that increasing ARWMC score was shown to decrease cerebral white matter integrity at frontal lobes, in specific myelin integrity. Age and cardiovascular risk factors are the predictors of ARWMC score. Increasing age and QRISK2 score were shown to increase the ARWMC score.

DISCLOSURE

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