GRADING OF SUPRATENTORIAL GLIOMAS USING MR DIFFUSION TENSOR IMAGING

DR. TAN KIA SING

Dissertation Submitted in Partial Fulfillment of the

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Supervisor

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To my beloved wife Niensi,

my adorable daughter Elise,

but not least my parents and siblings.

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ABBREVIATIONS

ADC	-	Apparent diffusion coefficient
AD	-	Axial diffusivity
Cho	-	Choline
Cr	-	Creatine
CT	-	Computed Tomography
DWI	-	Diffusion weighted imaging
DTI	-	Diffusion tensor imaging
EGFR	-	Epidermal growth factor receptor
EPI	-	Echo planar imaging
FA	-	Fractional anisotropy
FLAIR	-	Fluid-attenuated inversion recovery
FOV	-	Field of view
GBM	-	Glioblastoma multiforme
GRE	-	Gradient echo sequence
HPE	-	Histopathological examination
HUSM	-	Hospital Universiti Sains Malaysia
IDH	-	Isocitrate dehydrogenase
IgE	-	Immunoglobulin E
IQR	-	Interquartile range
MD	-	Mean diffusivity
MR	-	Magnetic Resonance
MRI	-	Magnetic Resonance Imaging
MRS	-	Magnetic Resonance Spectroscopy

NAA	-	N-acetylaspartate
NAWM	-	Normal-appearing white matter
PACS	-	Picture Archiving Communication System
ppm	-	parts per million
PWI	-	Perfusion weighted imaging
RA	-	Relative anisotropy
RAI	-	Relative anisotropy index
RD	-	Radial diffusivity
ROC	-	Receiver operating characteristic
ROI	-	Region of Interest
SD	-	Standard deviation
SENSE	-	Sensitivity-encoded
SPSS	-	Statistical Package for Social Sciences
TE	-	Echo time
TI	-	Inversion time
TR	-	Repetition time
USA	-	United States of America
VEGF	-	Vascular endothelial growth factor
WHO	-	World Health Organisation

ABSTRAK (BAHASA MELAYU)

Topik: Penggredan Glioma Supratentorial Menggunakan Pengimejan Resonans Magnetik (MR) Difusi Tensor (DTI)

Latar Belakang dan Tujuan: Gliomas merupakan tumor otak utama yang paling biasa yang kebanyakannya terletak di kawasan supratentorial pada orang dewasa. Mereka dikelaskan kepada gred rendah (gred I dan II) dan gred tinggi (gred III dan IV) berdasarkan klasifikasi WHO. Pengimejan MR konvensional adalah penting untuk mencirikan morfologi tumor tetapi kekurangan ketepatan dalam menentukan gred tumor. Teknik canggih MR seperti DTI telah muncul sejak beberapa dekad lalu sebagai kaedah tambahan untuk menilai secara mendalam tumor otak di peringkat mikrostruktur dan fisiologi.

Penggredan tepat glioma adalah penting untuk menentukan cara rawatan dan urutan pengurusan untuk pesakit. Oleh itu, kajian ini bertujuan untuk secara retrospektif menentukan sama ada nilai-nilai FA atau ADC pada 3-Tesla MR DTI jauh berbeza antara gred rendah dan gred tinggi glioma supratentorial. Nilai-nilai FA dan ADC antara glioma dan hemisfera serebrum yang normal juga dibandingkan.

Metodologi: Enam belas pesakit yang berumur 18 tahun dan ke atas yang baru didiagnosa glioma supratentorial dengan keputusan histopatologi dimasukkan dalam kajian. MRI mereka dengan turutan DTI diambil dari PACS

ke dalam program “MR extended workspace” untuk analisis data. Data DTI telah didaftar bersama dengan imej T1 selepas administrasi gadolinium. Empat kawasan kajian (ROI) dilukis pada margin depan, belakang, sisi dan medial tumor. ROI juga diletakkan di sumsum otak putih kontralateral lobus yang bertentangan. Perbandingan nilai-nilai FA dan ADC antara glioma gred rendah dan gred tinggi, dan di antara glioma dengan sumsum otak putih kontralateral telah dilakukan dengan menggunakan ujian Mann-Whitney U dan ujian Wilcoxon signed ranks.

Keputusan: Terdapat perbezaan yang signifikan secara statistik antara nilai FA glioma gred rendah dan gred tinggi, dan di antara nilai-nilai FA dan ADC daripada glioma dan sumsum otak putih kontralateral ($p < 0.05$). Tiada perbezaan yang signifikan secara statistik antara ADC glioma gred rendah dan gred tinggi ($p = 0.129$).

Kesimpulan: Nilai FA dijana daripada DTI boleh digunakan untuk penggredan glioma. Nilai ADC daripada glioma gred rendah adalah lebih tinggi daripada glioma gred tinggi tetapi tidak signifikan secara statistik.

ABSTRACT (ENGLISH)

Topic: Grading Of Supratentorial Gliomas Using MR Diffusion Tensor Imaging

Background and Purpose: Gliomas, being the most common primary brain tumours are usually located at the supratentorial regions in adults. They are classified into low grade (grade I and II) and high grade (grade III and IV) based on WHO classification. Conventional MR imaging is essential to characterise the tumour morphology but lack accuracy in determining the grade of tumours. Advanced MRI technique such as DTI has emerged over the past decades as an additional method to further evaluate the brain tumours at the microstructural and physiological levels.

Accurate grading of gliomas is important to determine the mode of treatment and sequences of management for patients. Therefore, this study aims at retrospectively determines whether FA or ADC values at 3-Tesla MR DTI are significantly different between low grade and high grade supratentorial gliomas. FA and ADC values between the gliomas and normal cerebral hemisphere are also being compared.

Methodology: Sixteen patients aged 18 years old and above with newly diagnosed supratentorial gliomas with histopathological results were included. Their MRI with DTI sequence were retrieved from PACS into extended MR workspace for data analysis. DTI data was co-registered with post-gadolinium

T1 weighted images. Four ROIs were drawn at the anterior, posterior, lateral and medial margins of tumours. ROIs were also placed at the contralateral NAWM of the opposite lobe. Comparison of FA and ADC values between low grade and high grade gliomas, and between gliomas with contralateral NAWM was performed using Mann-Whitney U test and Wilcoxon signed ranks test respectively.

Results: There was significant difference between FA of low grade and high gliomas, and between FA and ADC of gliomas and contralateral NAWM ($p < 0.05$). No statistical significant difference was found between the ADC of low grade and high grade gliomas ($p = 0.129$).

Conclusion: FA values generated from DTI can be used to grade gliomas. ADC values of low grade gliomas are higher than that of high grade gliomas but are not statistical significantly different.

CHAPTER 1 INTRODUCTION

Primary brain tumours in adults are commonly found in the supratentorial region. Whether they are extra-axial meningiomas or intra-axial gliomas, they are graded into low grade (grade I or II) or high grade (grade III or IV) based on World Health Organisation (WHO) classification.

Magnetic resonance imaging (MRI) over the years has become the imaging of choice for evaluation and characterisation of brain tumours, due to its superiority in exhibiting the soft tissue contrast as compared to computed tomography (CT). Establishing the diagnosis of brain tumours and the accurate grading is crucial, as it would largely affect the decision-making with regards to the treatment, whether patient can be managed conservatively or whether surgical removal is required (Essig et al., 2012).

Conventional MR imaging with gadolinium-based contrast agents is helpful in providing information regarding the tumour enhancement pattern, perilesional oedema, haemorrhage, necrosis and mass effect. However, several studies have shown that this information may be inaccurate in determining the grading of the tumours despite optimisation of the sequences and protocols (Law et al., 2003).

With the advancement of technology over the recent years, advanced MR imaging techniques such as diffusion tensor imaging (DTI) has emerged as a method to better assess and characterise the brain tumours.

Diffusion tensor Imaging (DTI) is a relatively new imaging technique, which relies on the ordered anisotropy diffusion of water along the white matter tracts. Water molecules show microscopic random (Brownian) translational motion, thus the molecular mobility of water is the same in all direction. This is termed ‘diffusion isotropy’. However in vivo, complex microstructures in tissue such as white matter tracts, capillary vessels and cell membranes act as barriers, causing directionality of water diffusion to arise in three-dimensional space. This directional variation is called ‘diffusion anisotropy’. DTI has the ability to quantitatively acquire the magnitude and directionality of water diffusion along a vector in a three-dimensional space. Several parameters have been used to quantify the anisotropy, the common ones being fractional anisotropy (FA) and apparent diffusion coefficient (ADC) (Beppu et al., 2003).

Brain tumours cause disturbance to the architecture and water content in the brain tissue. Such disturbance in the cell structure will change the magnitude and directionality of water diffusion, of which DTI is able to capture and quantify. This may potentially more accurately assess the brain tumours than just conventional MRI. Even though previous studies on MR spectroscopy, conventional diffusion-weighted imaging and perfusion MRI have suggested that these functional studies are helpful in glioma grading,

DTI for glioma grading remains an area still fresh for more exploration and research (Lee et al., 2008).

The aim of this study is to therefore retrospectively determine whether FA or ADC value at 3-Tesla MR DTI is significantly different between low grade and high grade supratentorial gliomas and its usefulness for glioma grading. Apart from that, the FA and ADC values between the gliomas and normal cerebral hemisphere are also being evaluated.

The rationale of this study is that accurate grading of the gliomas is essential in determining the mode of treatment and sequences of management for the patients. Even though conventional MRI can provide the morphological and anatomical features, it cannot reliably grade the tumours, as the features between the low grade and high grade gliomas can overlap. Thus, by adding the DTI sequence and evaluating the parameters, which include FA and ADC, it is aimed to determine a reproducible way of drawing the region of interest (ROI) to differentiate the low grade and high grade gliomas.

CHAPTER 2 LITERATURE REVIEW

Brain tumours compared to other tumours are relatively uncommon, accounting for 2.7% and 1.7% respectively of all cancers in males and females in Peninsular Malaysia (Lim et al., 2008). Having said that, they are the most debilitating tumours due to their location in the brain. Even benign brain tumours can cause significant functional impairment or even death. Approximately half of the patients with brain tumours still survive one year after the diagnosis (Lee *et al.*, 2014a). With the recent advancement in neuroimaging technology, the management of patients with brain tumours have improved owing to early accurate diagnosis and localization.

2.1 Glioma

The incidence of central nervous system tumours in the United States in 2000 was 6.7 per 100,000 persons and gliomas account for approximately 51% of all cases (Hess et al., 2004). This is much higher as compared to the incidence in HUSM, which is 35% of all intracranial tumours. Glioma incidence is slightly higher among males, accounting for 57.4% of all gliomas (Yusof, 1998). The incidence has been on the increasing trend over the years, due to the fact that development of diagnostic imaging is rampant in the past decade. Others have attributed this to the changes in classification and coding system (Vovoras et al., 2014).

Glioma arises from abnormal proliferation of glial cells. The nervous system is made up mainly by two groups of cells, namely neurons and glial cells. Glial cells are non-neuronal cells that surround neurons and function by formation of myelin sheaths around axon. They also help to maintain the optimal concentrations of ions and neurotransmitters in the neuronal environment. In response to injury, they are responsible for the regulators for neuronal repair. Glial cells are in fact a broad category of cells made up of many sub-types (Jessen, 2004). Thus, gliomas can be classified based on their cell types and are subdivided into astrocytomas, oligodendrogliomas, ependymomas, brainstem gliomas, optic nerve gliomas and mixed gliomas. More than 75% of patients diagnosed with gliomas consist of astrocytomas. Astrocytomas are graded using standard WHO scheme that depends on assessment of nuclear atypia, mitotic activity, cellularity, vascular proliferation and necrosis. They are classified into four grades, namely pilocystic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma multiforme (GBM) (grade IV).

The loss of tumour suppressor genes and activation of oncogenes are two established fundamental mechanisms that cause cancer in humans, including gliomas. In particular, glioblastoma multiforme has loss of genetic materials on chromosome 10 and epidermal growth factor receptor (EGFR) gene amplification (Andreas von Deimling et al., 1992). Gliomas may be associated with certain inherited syndromes such nuerofibromatosis type 1 and 2, Turcot's syndrome and Li-Fraumeni syndrome, but the incidence is very rare (Malmer et al., 2001). The only most established and proven

environmental risk factor to cause increased risk of gliomas is therapeutic ionising radiation. Particularly in children who have been treated with therapeutic irradiation for acute lymphoblastic leukaemia have significant increased risk of developing gliomas and primitive neuroectodermal tumour (PNET) within 10 years after therapy. It was found that the mutations of TP53 occurred among these patients, causing the formation of low grade gliomas and glioblastomas (Ohgaki and Kleihues, 2005). Association with head injury, food containing N-nitroso compounds, occupational risk, exposure to electromagnetic fields and usage of mobile telephones have no strong evidence of increased risk of gliomas. On the other hand, association has been found between immunologic factors and gliomas, for which patients with atopy have reduced risk of gliomas and those who have glioblastomas but with elevated IgE survive longer than those with normal levels (Wen and Kesari, 2008).

2.2 Clinical Presentation of Glioma

Patients with gliomas can virtually present with any neurological symptoms, depending on the location of the tumour rather than the histological type of the tumours (Schneider et al., 2010). Having said that, the commonest presentation is headache in up to 50% of patients, with non-specific pain pattern. Features such as progressive severity, new-onset headache and unilateral localisation in patients more than 50 years old are more suggestive of tumour-associated headache than just a benign headache (Omuro and DeAngelis, 2013).

Other clinical presentation would be partial or generalised seizures, in which it is frequent when the tumour is located at the cortex and is the slow growing type. Patients may also present with features of raised intracranial pressure, which include progressive headache, nausea, vomiting, drowsiness, visual disturbances (papilloedema on fundoscopy and diplopia secondary to abducens nerve palsy) and hydrocephalus due to cerebrospinal fluid circulation obstruction. These usually occur in rapidly growing tumours at the silent areas of the brain (right frontal and temporal lobes), causing vasogenic oedema. Progressive neurological deficit is another possible clinical presentation, and it helps in determining the possible tumour site. Supratentorial tumours will cause motor or sensory deficits, hemianopia, aphasia or a combination of all these, while posterior fossa tumours will have symptoms and signs of cranial nerve palsies, cerebellar dysfunction and long-tract signs. Finally, patients may have variable severity of cognitive dysfunction, especially those with tumours at frontal lobes, meningeal spread of the tumour and diffuse brain infiltration (Behin et al., 2003).

Due to the variable presentations, clinical features are unreliable to determine low grade gliomas from high grade gliomas and other intracranial space occupying lesions. Thus, it is essential to perform neuroimaging to further evaluate these tumours.

2.3 Imaging of Glioma

Radiological imaging techniques have been advancing by leaps and bound over the recent years and diagnosis of the brain tumours has improved tremendously with the new imaging techniques. CT and MR are the two main modalities that play an essential role in neuroimaging (Zelenak et al., 2013). They are considered the primary non-invasive option to evaluate patient suspicious of brain tumours.

CT of the brain is acknowledged as an initial screening method for the patient suspicious of brain tumour. It is more easily available at most of the hospitals as compared to the MRI machine and is less time-consuming. Thus, it is especially useful in emergency setting when patient is not so stable and uncooperative, where a MR imaging protocol is impractical on the day of presentation (Essig et al., 2012). CT scan can be useful to rule out other pathologies that may present similarly as brain tumours, such as cerebral abscess, which warrant urgent loading of antibiotics and early surgical drainage if indicated. While CT scan has lower resolution than MRI, it is better in depicting calcification, bone abnormalities and hyperacute bleed. The setbacks of CT scan include possibility of missing some small tumours due to lower soft tissue contrast, moderate specificity in determining the types of tumours and exposure to ionising radiation.

The basic CT examination of brain tumours encompasses standard non-contrasted and contrast enhanced imaging (Zelenak et al., 2013).

Calcification within tumours can be evaluated on the non-contrasted images. Pattern and degree of enhancement on the other hand may help to arrive to a possible diagnosis. Other important features to note include mass effect that causes hydrocephalus and brain herniation, in which emergency extraventricular drainage and decompression are needed prior to a definitive surgery later.

MR imaging is a better option for further characterisation of cerebral gliomas. Conventional MR imaging is far superior than the CT scan due to higher sensitivity, higher soft tissue contrast to portray the anatomical conditions of the brain tissue, better depiction of the extent of tumours and the ability to have multiplanar acquisition (Schneider et al., 2010). MR scanner also produces little or no signal to the cortical bone structures, thus eliminating artefact. In contrast to CT scan, the posterior fossa and base of skull are difficult to be evaluated due to the beam hardening artefact produced by the bone (Oot et al., 1988).

The basic MR imaging protocol for the demonstration of the cerebral tumour include T2- and T1-weighted native and contrasted sequences, and fluid-attenuated inversion recovery (FLAIR) sequences. Certain cases may need to include T2-weighted gradient echo sequence (GRE) to show calcification and blood. In general brain tumours are hypointense or isointense on T1-weighted sequence and hyperintense or isointense on T2-weighted imaging. The tumour's signal is altered based on the intralesional proportion of the individual components, which include solid, cystic, necrotic or

haemorrhagic components, fatty tissue, or even increased proportion of protein in the cystic part (Zelenak et al., 2013).

In certain cases, the brain tumours can be hard to visualise in non-contrast imaging, thus the administration of a paramagnetic contrast medium is advocated. Intravenous contrast medium is administered in order to reveal the full extent of the tumour and to differentiate the tumour tissue from perilesional oedema, between viable and non-viable tumour tissue, as well as detection of any small satellite lesions (Schneider et al., 2010).

2.4 Advanced MRI Techniques

Several earlier studies had shown that grading of gliomas with conventional MR imaging based on the tumour features have been erroneous, with sensitivity in identifying high-grade gliomas between 55.1% to 83.3% (Law et al., 2003). Tumour features include crossing of the midline, oedema, heterogeneity, haemorrhage, border definition, cyst or necrosis and mass effect. Two of these features are statistically significant predictors of tumour grading. Mass effect and necrosis tend to be more predictive of high-grade gliomas while other features are not statistically significant between both low- and high-grade gliomas (Dean et al., 1990). Another study revealed that there is high false-positive rate of 50% to diagnose low grade astrocytomas based on conventional MR imaging alone (Kondziolka et al., 1993).

With all these limitations, modern advanced MR imaging techniques have been evolved by leaps and bounds over the past decade. Neuro-diagnostic imaging is no longer confined to the assessment of pathological-anatomical condition, but also expands to evaluate the changes at microvascular integrity, haemodynamic characteristics, and the freedom of water molecule movement. In addition to that, the metabolic and biochemical changes are also being examined (Al-Okaili et al., 2006). Incorporating advanced MR imaging techniques, such as spectroscopy (MRS), diffusion weighted imaging (DWI), diffusion tensor imaging (DTI) and tractography, perfusion weighted imaging (PWI) and functional imaging (fMRI), will improve diagnostic, prognostic and therapeutic assessments. Not only can MR imaging help to evaluate structural abnormality and identify tumour-related complications, it also can aid in grading gliomas, in guiding stereostatic biopsies, differentiate types of tumours from primary to metastatic tumours, monitor treatment response and distinguish tumour recurrence from treatment necrosis, and eliminate tumour-mimicking lesions such as stroke and tumefactive-demyelinating lesions (Young and Knopp, 2006).

MR spectroscopy (MRS) is one of the advanced MR imaging technique that has moved from just clinical research level to become part of the routine clinical practice. MRS evaluates the biochemical and metabolic changes of the brain tumours and their surrounding tissues at the cellular level. Thus, it can discover the pathological process before the conventional method. Metabolic signals are obtained and expressed as a spectrum in which position of a specific metabolite is placed on the horizontal axis in chemical shifts

specified in parts per million (ppm) and the vertical axis represents the intensity of its signal (Zelenak et al., 2013). Typical metabolites being evaluated are choline (Cho), creatine (Cr), N-acetylaspartate (NAA), lipid, lactate and myo-inositol. NAA are predominantly found in neurons, thus reduced in its signal in tumours because the normal neurons have been destroyed by the neoplastic process. Choline on the other hand is elevated in neoplasm due to the increased cell membrane turnover caused by tumour growth. Elevated lactate signal is contributed by the relative anaerobic environment of many neoplasms and the incomplete breakdown of glucose. Despite that, there is no clear cut off metabolic signal ratios that can distinguish between neoplastic and non-neoplastic conditions. Prior publications on MRS showed that Cho/NAA ratio of greater than 1 is an indicator of neoplastic process with the sensitivity of 79% and specificity of 77%. Cho/NAA cut off ratio of 2.2 was reliable in distinguishing high grade tumours from low grade tumours and non-neoplastic conditions. In the area of grading primary cerebral tumours, high grade tumours are prone to have elevated lipid signal while low grade tumours have high myoinositol peak (Al-Okaili et al., 2006). A later study of using MRS in tumour grading revealed that the choline/NAA ratios are higher and the NAA/creatine ratios lower in high grade gliomas than in low grade gliomas. MRS is also able to determine that Cho/NAA and Cho/creatine are higher in high grade gliomas than low grade gliomas in the peritumoural hyperintensity, which is either vasogenic oedema or infiltrative tumour or both in conventional MR imaging. Thus, MRS helps in delineating the tumour extension better than the conventional MRI (Leung et al., 2014).

Diffusion-weighted imaging (DWI) employs the theory of diffusion that water molecules move in constant, random Brownian motion in all directions. Biological tissues are isotropic when the diffusion is the same in all directions and anisotropic if the diffusion is restricted in one direction, such as by cell membrane. In the brain, cerebrospinal fluid is an isotropic field. Grey matter is more limited than fluid but its diffusion is still in all directions and is therefore isotropic as well. White matter on the other hand is anisotropic because the diffusion progresses with greater intensity in the direction within axons (Zelenak et al., 2013).

DWI is acquired using motion-sensitive sequence; usually echo planar imaging (EPI) in three orthogonal directions, for the movements of protons in water. A proton will spin at the same rate under the same magnetic field, but when a pulsed gradient is applied, it will spin at different rate based on the strength, duration and direction of the gradient. The proton will then refocus when a second pulse gradient is being applied. If the protons have not moved the refocusing will be complete and the signal remains unchanged, while if the protons have moved between these two pulses, loss of signal that is dependant on the degree of diffusion weighting (referred to as b-value) and diffusion coefficient occurs. As the signal change with DWI is also dependant on the underlying T2-weighted signal, it cannot be used to quantify diffusion processes alone. Thus, the gradient of the signal intensity is plotted based on different b-values. This measure of all motional processes is called the apparent diffusion coefficient (ADC), which often mirrors the changes in DWI

(Price, 2007). Below is the example of DWI and ADC maps acquired in a patient with grade III anaplastic astrocytoma at the left frontal lobe (Figure 2.1).

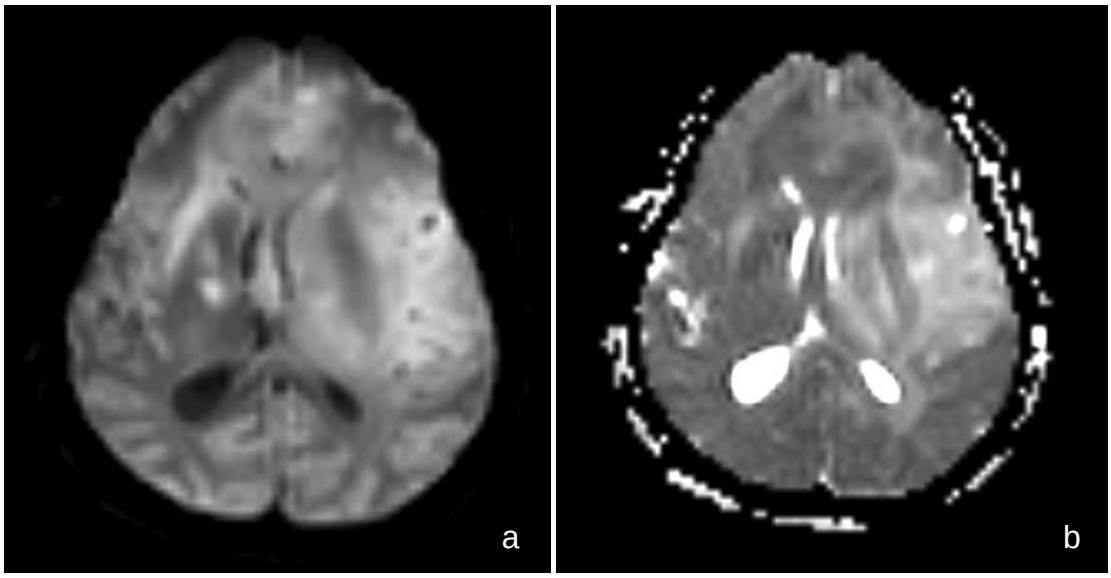

Figure 2.1 **DWI (a) and ADC (b) maps of a patient with left frontal anaplastic astrocytoma showing the reciprocal signal intensity of both maps.**

The application of DWI in neuroimaging is vast, which encompasses the evaluation of stroke, cerebral infections, cerebral tumours, traumatic changes and demyelinating lesions. Specifically in the areas of cerebral tumours evaluation, previous studies have shown that ADC maps can help to differentiate tumour, oedema, cysts and necrosis, thus being a powerful tool to characterise cerebral tumours (Castillo et al., 2001). In one study regarding the role of DWI in patients with brain tumours, the authors found that ADC values cannot reliably differentiate types of tumours, but lower ADC values indicate high grade gliomas while higher ADC values represents low grade astrocytoma. ADC values in this study correlated well with the tumour cellularity in astrocytomas and meningiomas (Kono et al., 2001). However, a later study particularly on ADC of human brain tumours at MR imaging, the authors concluded that ADC is able to differentiate some of the brain tumours in humans. They discovered a good inverse correlation between ADC and WHO grade II to IV astrocytic tumours, whereby the accuracy to differentiate grade II from grade III & IV is 91.3% and the accuracy to differentiate grade III from grade IV is 82.4% (Yamasaki et al., 2005).

Another advanced MR imaging that also utilises the diffusion of water is diffusion tensor imaging (DTI), which is a technique that has been developed more recently than the DWI. The difference between these two techniques is that DTI will sample the water movement in minimally six non-collinear directions, as compared to DWI which acquire the movement in three directions only (Provenzale et al., 2006). DTI is sensitive to anisotropic or directionally dependent diffusion, thus providing unique information on three-

dimension (3D) diffusivity, characterised by three eigenvectors (direction) and three eigenvalues (magnitude). The term “tensor” is a mathematic construct derived from physics and engineering to describe tension forces in solid bodies with an order of 3D vectors. Tensor is formed from a matrix of numbers adopted from diffusion measurements in at least six independent diffusion-encoding directions to calculate orientation-dependent diffusion in all spatial directions for each voxel in DTI. In human brains, the anisotropic diffusion is mainly contributed by myelin and axons. The unique microscopic diffusion barrier created by the lipid bilayer of myelin sheath results in various degrees of diffusion along different planes. Despite that, this alone cannot be responsible entirely for the anisotropic diffusion as non-myelinated nerves also show significant diffusion anisotropy. Therefore, the axons and their subcomponents such as microfilaments, neurofilaments, microtubules and membranes also influence the diffusion anisotropy greatly. Despite the biologic basis of diffusion anisotropy is yet to be completely understood, this unique features caused by the axonal bundles and myelin sheath enable the measurement and fibre orientation tracking, especially in the large white matter tracts, thus helping tremendously in neuroimaging and neurosurgery (Cha, 2006).

The DTI data needs to be extracted and be analysed in three ways to provide information on tissue microstructure and architecture for each voxel. First is the mean diffusivity, which characterises the overall mean-squared displacement of molecules and presence of restriction to diffusion. Secondly, the degree of anisotropy, which describes the amount of molecular

displacements vary in space and is related to the oriented structures. Thirdly, the main direction of diffusivities that is connected to the orientation in space of the structures. Thus, the most common derived DTI parameters are fractional anisotropy (FA) and mean diffusivity (MD). Anisotropy is usually represented by the FA or alternatively by the relative anisotropy (RA). The FA is a measure of the fraction of the magnitude of the diffusion tensor owing to anisotropy, while RA is composed by the ratio between the anisotropic and isotropic portions of the diffusion tensor. FA ranges from 0 to 1, in which 0 represents isotropic with zero net direction and 1 denotes maximal anisotropy that occurs along the primary eigenvector. MD on the other hand is the mean of the three eigenvalues or a directionally averaged measure of the magnitude of water diffusion. MD is analogous to ADC and is related to the integrity of the brain tissue (Le Bihan et al., 2001).

The most common clinical application of DTI is in the area of tractography. Tractography derived from DTI data maps allows visualisation of the white matter tracts, thus allowing neurosurgeon to know the topography, integrity and degree of involvement of the white matter fibres in pre-operative planning. This would help in minimising the functional damage and determining the extent of pathologic tissue infiltration in order to minimise residual tumour volume. White matter tracts are characterised as displaced when they maintain normal anisotropy relative to the corresponding contralateral tract but are abnormally located or orientated based on the standard colour code. They are considered as oedematous if they maintain the normal anisotropy and orientation but with obvious high signal intensity on

T2 weighted images. If the white matter tracts have reduced anisotropy yet remain visible on the orientation map, they are considered as infiltrated by tumour. Lastly, they are termed degenerated when the anisotropy is significantly reduced such that no identifiable tracts are seen on the orientation maps (Zoccatelli et al., 2013). The below figure is an example of a DTI map of a patient with right parietal pilocystic astrocytoma (Figure 2.2).

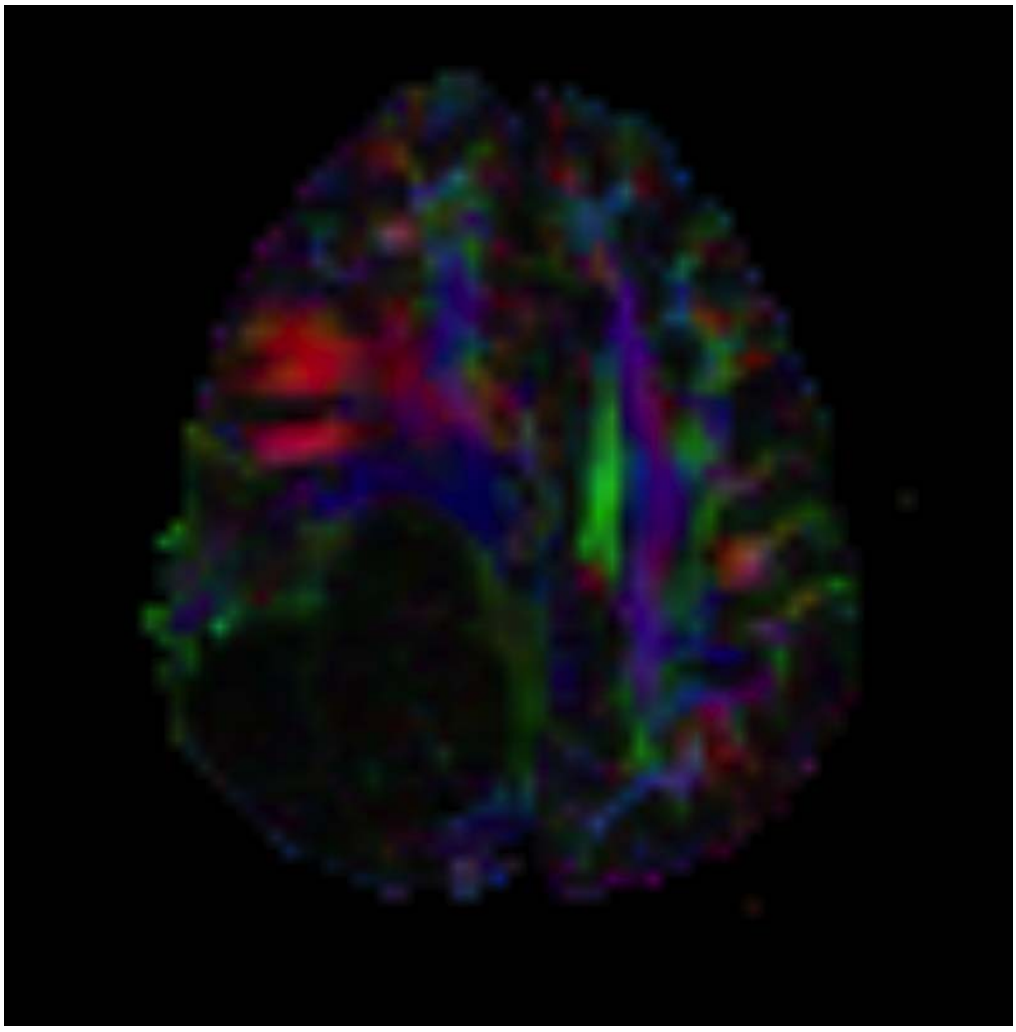

Figure 2.2 DTI map of a patient with right parietal pilocystic astrocytoma showing disruption of the normal white matter colour code.

Apart from that, many studies have been centred upon characterising and grading brain tumours using DTI in order to establish more applications of DTI. In a study of peritumoural DTI of high grade gliomas and metastatic brain tumours, both displayed significant increases in MD and significant decreases in FA when compared with normal-appearing white matter (NAWM). The peritumoural MD of metastatic brain tumours is also significantly greater than that of gliomas, but not the FA values. Thus this concluded that diffusion metrics are altered within the vasogenic oedema around the gliomas and metastatic brain tumours, reflecting increased extracellular water. And peritumoural MD can be used to distinguish high grade gliomas from metastatic brain tumours (Lu et al., 2003).

Another study on using DTI for glioma grading at 3-Tesla MRI revealed that FA ratios at the non-enhancing regions were not significantly different between low and high grade gliomas, but MD ratios were significantly lower in high grade gliomas. And in high grade gliomas, the enhancing tumours showed lower FA ratios than the non-enhancing tumours. Thus, the author concluded that MD values of non-enhancing gliomas are useful for grading (Lee et al., 2008).

In a study of measurement of FA using DTI in supratentorial astrocytic tumours, the author stated the mean FA values of frontal or occipital lobes differed significantly with genu and splenium of corpus callosum, but the differences between the frontal lobe and occipital lobe or between the genu and splenium were not significant. Mean FA value of frontal lobe was 0.33 ±

0.04, occipital lobe 0.32 ± 0.04, genu 0.69 ± 0.04 and splenium 0.72 ± 0.04. Regardless of the histological types of the astrocytomas, the mean FA values were lower than that of normal appearing white matter. The findings in the study suggested the FA value of an astrocytic tumour is influenced by the balance between destruction or displacement of normal fibre structure and cellularity and/ or vascularity. Thus, measurement of FA value using DTI will predict the histological characteristics (Beppu et al., 2003).

A study about the DTI of high grade cerebral gliomas focused on differentiating tumour core, oedematous brain and normal-appearing white matter using DTI. In this study, it was found that the MD was highest in the necrotic tumour core (1825.3 x 10^{-6} mm^2/s), followed by oedematous brain (1411.23 x 10^{-6} mm^2/s), enhancing tumour core (1308.67 x 10^{-6} mm^2/s), enhancing tumour margin (1229.80 x 10^{-6} mm^2/s) and normal brain (731.53 x 10^{-6} mm^2/s), out of which MD was significantly different in enhancing tumour margins and oedematous brain in all patients. FA on the other hand was highest in the normal brain (0.47 ± 0.08), followed by oedematous brain (0.17 ± 0.08), enhancing tumour margin (0.16 ± 0.06), enhancing tumour core (0.13 ± 0.06) and lowest in necrotic tumour core (0.09 ± 0.03). This is however significantly different in only seven out of the nine patients (Sinha et al., 2002).

One of the latest studies in evaluating the FA characteristics of cerebral gliomas using DTI used not just mean FA, but also maximum and minimum FA values, FA range as well as maximum standard deviation (SD). The study revealed that the maximum FA, FA range and maximum SD for

grade II tumours were significantly lower than those for grade III and IV tumours. A very good correlation of maximum FA to FA range and maximum SD were obtained, therefore suggesting that FA range and maximum SD to be the added values to findings on conventional imaging in differentiating low and high grade gliomas (White et al., 2011). However, the placement of region of interest (ROI) for maximum FA and maximum SD can be tedious and difficult.

2.5 Histopathological Criteria for Grading of Gliomas

The fourth edition of the World Health Organisation (WHO) of tumours of the central nervous system was published in 2007 with several new entities being listed. The very first international classification of human tumours was initiated through a resolution of the WHO Executive Board in 1956 and the World Health Assembly in 1957. The main aim for this was to establish a classification and grading of human tumours that is accepted and used worldwide. Thus, first edition on histological typing of tumours of the nervous system was edited by Zulch and published in 1979. The second edition followed due to the advances in immunohistochemistry being introduced into diagnostic pathology. The third edition edited by Kleihues and Cavenee and published in 2000, incorporated genetic profiles in defining brain tumours. It also included epidemiology, clinical signs and symptoms, imaging, prognosis and predictive factors (Louis et al., 2007).

It was not known whether gliomas originate from mature or neuroectodermal stem cells located in the adult brain, but pathological diagnosis is dependent on similarities of the tumour cells to non-neoplastic mature glial cells. According to WHO classification, the three main types are astrocytomas, oligodendrogliomas, and mixed astrocytomas. Regardless of the histological cell types, gliomas are graded into low grade and high grade based on four main features: nuclear atypia, mitoses, microvascular proliferation and necrosis (Behin et al., 2003).

Low grade tumours include grade I and II tumours, while high grade tumours include grade III and IV tumours. Specifically, grade I tumours are lesions with low proliferative potential and cure is possible following surgical resection. Grade II lesions encompass tumours that are infiltrative in nature, and despite having low proliferative activity, often recur. Some grade II tumours may undergo malignant transformation into higher grades of tumours, for instance low grade diffuse astrocytoma that transform into anaplastic astrocytoma or glioblastoma. Neoplasms designated grade III are lesions with histological features of malignancy, namely nuclear atypia and brisk mitotic activity. Patients with grade III tumours usually receive adjuvant radiotherapy and/or chemotherapy. Anaplastic astrocytomas (WHO grade III) account up to 15% of malignant gliomas and the prognosis post-operation is about two years. Grade IV tumours are assigned when they are cytologically malignant, with active mitosis and necrosis, widespread infiltration of surrounding tissues as well as propensity for craniospinal spread (Louis et al., 2007). Glioblastoma multiforme represents most of the grade IV tumours, accounting

up to 52% of all gliomas. The peak incidence is at the fifth to sixth decades and the mortality is approaching 100% with the median survival of seven months (Hess et al., 2004).

Despite histological diagnosis is the gold standard to diagnose gliomas, several studies have provided evidence of clinically significant interobserver variation on the typing and grading of gliomas. Erroneous classification and grading would lead to both undertreatment and overtreatment, as the treatment decisions and patient care are dependent upon the histological typing and grading. The occurrence of interobserver variation is largely due to technicalities, whereby the pathologists are not reviewing the exact same material due to sampling error, for which only very tiny fragments or a few slides were submitted. Another reason for the variation would be how the tumours are classified. Several subtypes of gliomas are being differentiated by their morphological appearance. The WHO classification utilises morphological description for the various histologies and grades, which contain subjective elements, for instance “moderately increased” cellularity for grade II astrocytoma and “increased” cellularity for grade III anaplastic astrocytoma. Due to the definitions, boundaries between grades and tumour types are subject to interpretation. More importantly, the dedifferentiation of low grade tumour into higher grade tumour is a gradual and continuous process, leading to artificial boundaries between grades. Tumour grades are not reflecting the real and existing different entities (van den Bent, 2010).

To date, apart from the histological diagnosis, molecular markers recently have been routinely acquired to aid in diagnosis and treatment guidance of gliomas. For instance, genetic loss on chromosomes 1p/19q is pathognomonic for oligodendroglioma and mutations of TP53 would suggest a astrocytic lineage. Mutations of the isocitrate dehydrogenase gene (IDH) 1 or 2 are characteristic of low grade gliomas. If they are found in high grade glioma, it would mean that the tumour has developed from a lower grade precursor lesion. These IDH-mutated tumours have more favourable prognosis (Stupp et al., 2014). Glioblastoma multiforme on the other hand would exhibit loss of genetic material on chromosome 10 and amplification of epidermal growth factor receptor (EGFR) in 72% and 38% of cases respectively (Andreas von Deimling et al., 1992).

2.6 Treatment of Glioma

The treatment of gliomas is highly individualised depending on the histological diagnosis and other factors, such as tumour grade, patient's Karnofsky performance score to assess the daily functionality, neurological deficits and age (Schneider et al., 2010). Specialised multidisciplinary team involving neurosurgeons, medical and radiation oncologists, expert neuropathologist and neuroradiologist should be formed to evaluate individual patients and to provide the best treatment plan (Stupp et al., 2014).

Management of glioma patients includes general management aim at symptomatic relief and specific management, which is definitive treatment,