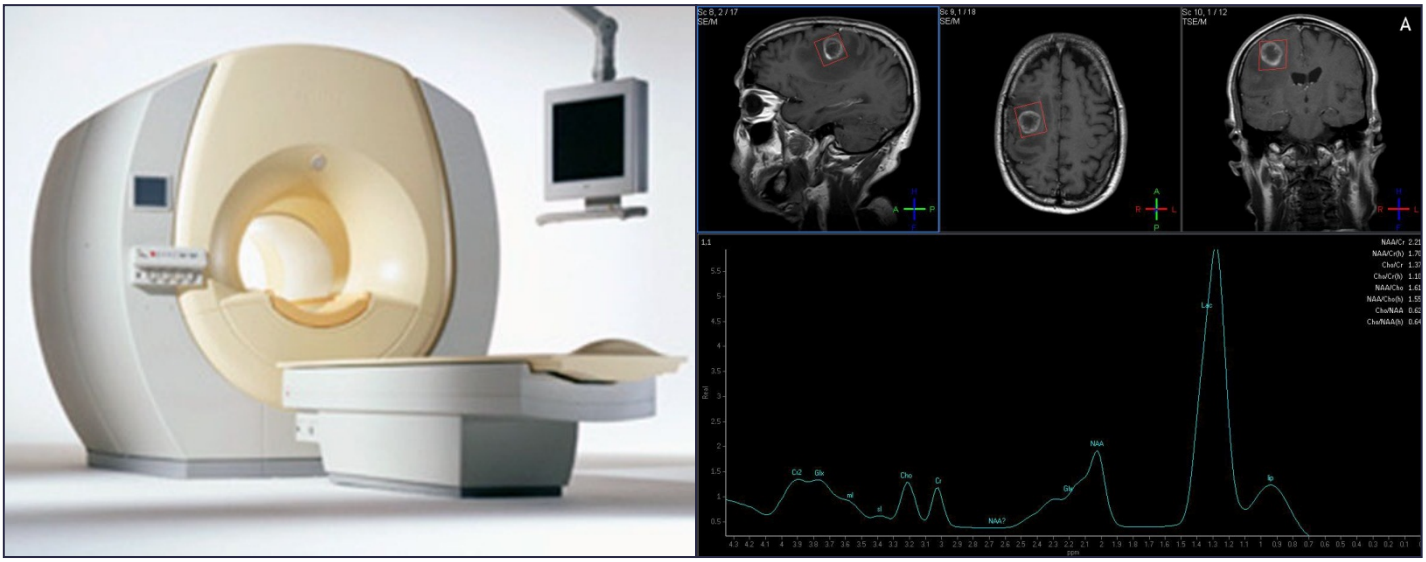
Clinical Application of Magnetic Resonance Spectroscopy in Diagnosis of Intracranial Mass Lesions

A Nairobi Outpatient Radiology Practice Perspective



Dissertation Submitted as Partial Fulfillment for the Degree of Master of Medicine in Diagnostic Imaging of the University of Nairobi

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Declaration

I, Dr. Mufaddal Nuruddin Wajihi, declare that this dissertation has not been submitted for any other degree in this or any other university or institution of higher learning and that the views expressed herein are my original work unless otherwise acknowledged.

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Approval by Supervisors

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Abbreviations

^{1}H	-	Proton of stable Hydrogen-1 atom (with 1 proton and 0 neutrons)
^{1}H-MRS	-	Proton Magnetic Resonance Spectroscopy
AA	-	Amino Acid
Ac	-	Acetate
ADEM	-	Acute Disseminated Encephalomyelitis
B_0	-	Static external magnetic field responsible for longitudinal magnetization
CHESS	-	Chemical-Shift Selective
Cho	-	Choline
CI	-	Confidence Interval
Cr	-	Creatine
CSI	-	Chemical Shift Imaging
CT	-	Computed Tomography
DICOM	-	Digital Imaging and Communications in Medicine
DWI	-	Diffusion Weighted Imaging
Dx	-	Diagnosis
EBM	-	Evidence Based Medicine
FID	-	Free Induction Decay
FLAIR	-	Fluid Attenuated Inversion Recovery
GABA	-	Gamma-Aminobutyric Acid
GBM	-	Glioblastoma Multiforme
Gd	-	Gadolinium
Glx	-	Glutamine/Glutamate/GABA peak
HIE	-	Hypoxic-Ischaemic Encephalopathy
HIV	-	Human Immunodeficiency Virus
IR	-	Inversion Recovery
JPEG	-	Digital image format named after "Joint Photographic Experts Group"
Lac	-	Lactate
Lip	-	Lipid
MHz	-	Megahertz
MR	-	Magnetic Resonance
MRI	-	Magnetic Resonance Imaging

MRS	-	Magnetic Resonance Spectroscopy
MS	-	Multiple Sclerosis
MT	-	Magnetization Transfer
M_{xy}	-	Transverse magnetization in the x-y plane
M_z	-	Longitudinal magnetization in the z plane
NAA	-	N-acetyl aspartate
NMR	-	Nuclear Magnetic Resonance
NMV	-	Net Magnetization Vector
PACS	-	Picture Archiving and Communication System
PCNSL	-	Primary CNS Lymphoma
PD	-	Proton Density
PML	-	Progressive Multifocal Leukoencephalopathy
PNET	-	Primitive Neuroectodermal Tumour
ppm	-	Parts per million
PRESS	-	Point Resolved Spectroscopy
RF	-	Radiofrequency
SI	-	Spectroscopic Imaging
S/N	-	Signal-to-Noise Ratio
STEAM	-	Stimulated Echo Acquisition Mode
Suc	-	Succinate
T	-	Tesla
T1	-	Time constant for longitudinal relaxation
T1w	-	T1 Weighted
T2	-	Time constant for transverse relaxation due to spin-spin energy transfer
T2*	-	Time constant for signal decay due inhomogeneities of external magnetic field
T2w	-	T2 Weighted
TE	-	Echo Time
TMS	-	Tetramethylsilane
TR	-	Repetition Time

Abstract

Introduction

Conventional MR imaging provides highly detailed anatomic information with unrivalled soft tissue contrast making it the mainstay in the diagnosis of suspected brain and spinal cord lesions. Despite this, MRI alone at times cannot answer the diagnostic questions in quite a few patients(1). Proton MR Spectroscopy (^{1}H-MRS) provides non anatomic information on the metabolic composition within an area of tissue under interrogation. By comparing the relative concentrations of specific metabolites, the neuroradiologist can deduce critical information regarding neuronal cell density and integrity, cell membrane turnover, metabolic fuel and possible necrosis in the region of interest(2). This provides a biochemical picture of the underlying pathology and thus aids in the differentiation among ischaemic mass lesions, intra- and extra-axial brain tumours, discrimination between high and low grade tumours, and discrimination between neoplastic and non-neoplastic lesions.

Study Objective

The objective of this study was to evaluate the clinical utility and diagnostic value of ^{1}H-MRS as an adjuvant to conventional MRI in the diagnosis of intracranial mass lesions in our local setup.

Study Design and Methodology

A total of 68patientswere referred to Plaza Imaging Solutions, Nairobi for brain MRS examinations from September 2012 to September 2013. A consecutive series of 63 patients' examinations which met inclusion criteria, were retrospectively studied. All patients were investigated under a constant single-voxel^{1}H-MRS PRESS protocol following structural MRI imaging on a 1.5T Phillips Intera MRI Scanner. All MRI and MRS examinations were reported by the attending radiologists. Data on observed MR spectra and metabolite ratios was analysed against the reported diagnoses. The data was analysed to determine the diagnostic value of ^{1}H-MRS added to MRI.

Results

Of the 63 patients examined by MRI and MRS for intracranial mass lesions, the radiologists were able to offer a single imaging diagnosis based on MRI alone in only 15 patients (23.8%) while when MRI imaging was combined with MR spectroscopy, a single imaging diagnosis was offered in 47 patients (74.6%). This was an overall statistically significant improvement of 313.4% (P-value <0.001)

The most notable indications, for which MRS aided the radiologist in offering a single diagnosis, were: high v/s low grade gliomas, high grade gliomas v/s tuberculomas, cerebral infarcts v/s low grade gliomas, and recurrent tumours v/s radiation necrosis.

MRS combined with MRI 'improved' the imaging diagnosis in more than half of all patients examined. MRS also improved the imaging diagnosis in more than half of the patients within all four major indication groups. The improvement was however not statistically significant. 'Improvement' was based on whether or not a single imaging diagnosis was obtainable after MRS in a patient where using MRI alone it was not.

MRS showed statistically significant value in differentiating low grade from high grade gliomas, with high grade gliomas having depressed Creatine, and increased Cho:Cr and Cho:NAA ratios>2.00.

Differentiating between tuberculomas and high grade gliomas was challenging as Choline increase was seen in all tuberculomas. Only lesions with Cho:Cr and Cho:NAA ratios <2.00 could be confidently diagnosed as tuberculomas. This resulted in only a modest 51.6% improvement in diagnostic performance of single-voxel MRS for this indication.

1. Introduction

Conventional MR imaging provides highly detailed anatomic depiction of the human body with unrivalled soft tissue contrast, and has become the mainstay in the diagnosis of suspected brain lesions(2, 3). Advances in MRI technology – including, but not limited to, Gadolinium enhanced imaging, diffusion weighted imaging, perfusion studies, and susceptibility weighted imaging – have improved the accuracy of MRI diagnoses considerably. In spite of these advancements and improved sensitivity, many brain lesions remain a diagnostic problem because of poor specificity(4). Proton MR Spectroscopy (^{1}H-MRS) provides complimentary information, specifically the metabolic composition of the tissue under interrogation. While no less than 200 metabolites are generated in the brain, we are blinded to most. Proton MRS however employs mainly seven metabolites which are metabolically important and easily detectable. These principal metabolites and what they represent include:

- Lipids (Lip) - *Products of brain destruction*
- Lactate (Lac) - *Product of anaerobic glycolysis*
- N-acetyl aspartate (NAA) - *Neuronal marker*
- Glutamine / GABA (Glx) - *Neurotransmitters*
- Creatine (Cr) - *Energy metabolism*
- Choline (Cho) - *Cell membrane marker*
- myo-Inositol (mI) - *Glial cell marker*

By comparing the relative concentrations of these metabolites, the neuroradiologist can deduce critical information regarding neuronal cell density and integrity, cell membrane turnover, metabolic fuel and possible necrosis in the region of interest, thereby, the likely underlying pathology(5). The acquisition of MR spectroscopy only requires the relevant additional software and pulse sequences; and an extended imaging time of only 10-12 minutes per patient. ^{1}H-MR spectroscopy is a useful non-invasive add-on to MR imaging.

Using conventional MRI, an experienced neuroradiologist can accurately propose the likely histological diagnosis in 70-90% of brain lesions based on a variety of rather indirect imaging criteria and clinical data(1, 2). These include age and clinical presentation, location, calcification, cyst formation, and contrast enhancement among others(6). In the remaining 10-30%, the differentiation of ischaemic mass lesions, intra- and extra-axial brain tumours, discrimination between high and low grade tumours, and discrimination between neoplastic and non-neoplastic lesions however, may be ambiguous if the diagnosis is exclusively based on these criteria(6).

In contrast to MRI, CT, and angiography, i.e. methods that provide structural data, proton magnetic resonance spectroscopy (^{1}H-MRS) provides non-anatomic information related to neuronal density and integrity, cell wall proliferation or degradation, energy metabolism(7), and necrotic transformation of brain and/or tumour tissues(8). Hence, in conjunction with anatomic imaging modalities, ^{1}H-MRS is playing an increasingly important role in the discrimination of a number of common neurological disorders such as stroke, epilepsy, multiple sclerosis, HIV, dementia, head injury and near drowning(6).

Conventional 1.5T MRI is available at the Kenyatta National Hospital (which is Kenya's main public referral hospital), and a few of the major private diagnostic imaging establishments in Nairobi and Mombasa. This is a significant improvement from only a few years back when there were only two low strength MRI scanners in the whole country and no public hospital had access to these services(9, 10). Today, MRS services in Kenya are only available at three private establishments, only one of them offering multi-voxel MRS. All other MRI providers, including the national hospital, do not provide MRS facilities. The greatest hurdle to the implementation of MRS in diagnosis is the lack of awareness among clinicians on the utility of MRS; not only in brain pathology, but also prostate and breast diseases.

2. Literature Review

2.1. Diagnostic Efficacy of MRS

2.1.1. Tumours

In the United States, adult incidence rate for primary brain tumours is approximately 25 per 100,000. Of these 33% were malignant. The incidence in paediatric population is much lower at 49 per 1,000,000; although a larger 65% were malignant(11).

The majority of brain tumours (more than 50%) are metastases from systemic malignancies. 10% to 30% of systemic malignancies metastasise to the brain(12, 13).

Moller-Hartmann et al reported that the addition of ^{1}H-MR spectroscopy information "statistically significantly increased the proportion of correctly diagnosed cases to 71% compared to 55% with MRI alone"(6).

According to Ando et al, adding proton MRS to standard MRI improved the sensitivity of diagnosing residual tumour and tumour recurrence from 86% to 100%(14). Traber et al successfully demonstrated that MRS had a sensitivity of 72% and specificity of 82% in differentiating tumour from radiation necrosis(15).

Astrakas et al showed an increase in sensitivity of 13% and in specificity of 8% of MRS versus MRI alone in diagnosis of high grade gliomas.(16).These studies have clearly demonstrated the benefit of using MRS as an adjunct to MRI, and can provide confirmatory diagnoses in those few patients where MRI alone did not.

On the other hand Devos et al claimed that in differentiating high grade gliomas from metastases, MRS did not have a statistically significant benefit over MRI.(17, 18).

However, in differentiating high grade astrocytomas from low grade astrocytomas, Herminghaus et al demonstrated a sensitivity and specificity of 95% and 93% respectively, which was a statistically significant increase in diagnostic efficacy of MRS over MRI.(19).

Burtscher et al also showed that MRS improved diagnostic accuracy in differentiating histologically infiltrative brain tumours (like gliomas and lymphomas), from well circumscribed tumours (like metastasis, germinomas and pineocytomas). This was because of MRS's ability to show pathologic changes outside the area of contrast enhancement in infiltrative processes. However, MRS was not able to separate the type of infiltrative or circumscribed tumour(20).

2.1.2. Tumours versus non-neoplastic lesions

In the differentiation of tumour versus non-neoplastic lesions, Mishra et al differentiated 52 histopathologically proved tumour cysts, abscesses, or benign cysts by using single voxel ^{1}H-MR spectroscopy and diffusion-weighted MR imaging. The authors reported the sensitivity and specificity of ^{1}H-MR spectroscopy to be 96% and 100% respectively. This compares favourably with diffusion weighted imaging where specificity remained high (100%), but sensitivity was diminished (72%). This again was significantly better than MRI alone, where sensitivity and specificity were only 86% and 79% respectively(21).

Lai et al in a small study of 14 patients concluded that Diffusion-Weighted Imaging (DWI) and ^{1}H-MRS are useful as additional imaging techniques for establishing the differential diagnosis between brain abscesses and cystic or necrotic brain tumours. DWI (which is routinely done in MRI of the brain) requires less imaging time and is more accurate than ^{1}H-MRS. However MRS is more useful for small peripheral lesions. There are conflicting reports in literature about using DWI alone to distinguish abscesses from cystic tumours. Lai et al postulated that combining DWI with MRS would improve this distinction because of the unique spectra of pyogenic abscesses(22).

2.1.3. Brain Abscesses

Brain abscess incidence is widely varied in literature, ranging from as low as 1% in developed countries, to as high as 8% in developing countries - of all intracranial masses(23). There is a definite link between high incidence of brain abscesses and poor socio-economic conditions. As such it is a public health issue, which is increasing especially in the setting of HIV(24).

The definitive diagnosis of brain abscess is usually easily made based on conventional imaging. However, in a small number of cases, it may not be possible to differentiate it from glioblastoma multiforme (GBM) and a metastasis with necrosis. In vivo proton MR spectroscopy has been found to be useful in the differentiation of brain abscess from other cystic mass lesions(22, 25).

MRS has also been shown to be able to differentiate pyogenic from tuberculous abscesses, and from other cystic tumorous conditions.

Tuberculosis is the world's leading cause of death due to a single infectious agent. Although curable, mortality is on the rise due to the HIV epidemic, and the emergence of multidrug resistant strains(26). Although most CNS Tuberculosis present as meningitis, the disease may present as tuberculomas. Their incidence has been reported to be as high as 50% of all intracranial masses, in the developing world.(25, 27). Although the risk of tuberculosis is higher in HIV infected patients, there is no concrete evidence to suggest a higher incidence of tuberculomas in this group(28).

Although it is possible to differentiate pyogenic abscesses from tuberculous abscesses by MR spectroscopy; as shown by Gupta et al; the need does not often arise. Diffusion Weighted Imaging (DWI) which is included in standard MR imaging, can often make that distinction(29).

2.1.4. Toxoplasmosis and Lymphoma

The radiologist's diagnostic dilemma is often an HIV infected patient who presents with a ring enhancing lesion. On normal MRI in such patients, toxoplasmosis and lymphoma can look quite similar. MR spectroscopy has been shown to be quite useful in this situation. Toxoplasmosis will show marked increase in Lipid/Lactate with decrease in Choline/NAA. Whereas in lymphoma, there will be a markedly increased Choline peak with minimal increase in Lipid/Lactate(30, 31).

2.1.5. Conclusion

Peer reviewed studies on the efficacy of MRS in neurological diagnosis have been mixed. A meta-analysis in 2006 by Hollingworth et al of several MRS studies concluded that current evidence on the accuracy of ^{1}H-MR spectroscopy in the characterization of brain tumours was promising(32). A Cochrane review by Cabrera et al in 2008, however, concluded that the data obtained on advanced magnetic resonance

imaging techniques (including MRS) "was not sufficient to recommend its use in patients with ischaemic, tumoural or demyelinating pathologies"(33). An important drawback on the many studies published regarding application of MRS in neurology is that many of these do not follow EBM (Evidence Based Medicine) criteria. In the west, health insurance providers do not currently support the adoption of MRS(34). For example in one policy last reviewed in May 2011, Aetna, one of the largest medical insurance providers in North America, considers MRS "experimental and investigational". It states that there have not been enough studies to provide evidence towards the efficacy of MRS. More importantly no clinical trial has demonstrated improved patient outcome in those diagnosed with MRI alone versus those with MRS. The consensus in the literature is that further studies are necessary to determine MRS's role in diagnosis and treatment planning in neurological diseases"(35).

2.2. Fundamentals of MRS

MRI employs the principle of nuclear magnetic resonance (NMR), a spectroscopic technique that scientists have used for decades to study the molecular composition of substances(36). The equipment required to perform NMR consists simply of a strong magnet and a radio transmitter and receiver (**Figure 1**).

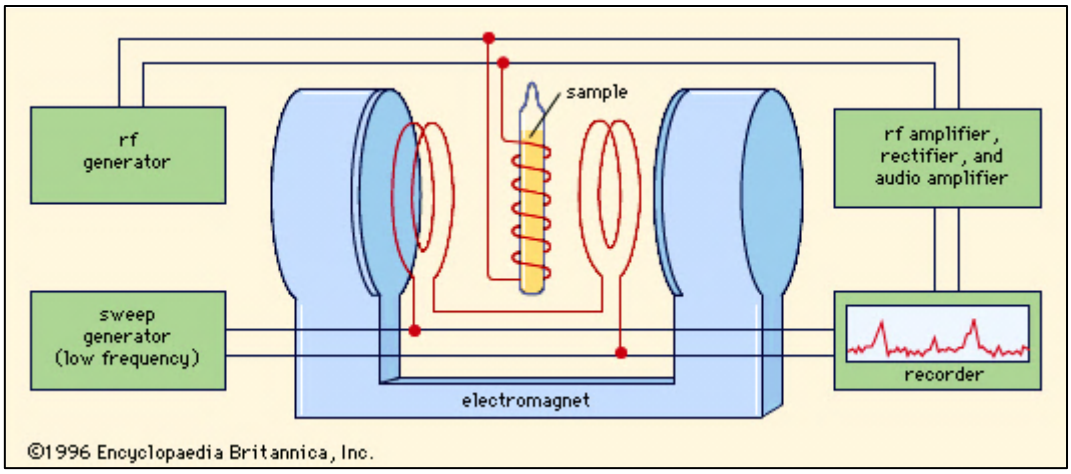


Figure 1: Diagram illustrating the equipment required to perform NMR Spectroscopy for conventional chemical analysis. ©*Encyclopaedia Britannica*

Whereas MRI utilises signals and combines them with spatial localization information to generate anatomic images(37), MRS uses signals from hydrogen atoms in molecular species at a particular point in the body to generate a spectroscopic plot which provides information on the relative amount of that molecular species (metabolites) present in that volume of tissue. MRS thus allows us to measure the chemical composition of the tissue being studied. These metabolites are dissolved in water and for them to be detectable by MRS, they need to present in concentrations of at least 1mMol(37). Proton (^{1}H) MRS is based on the "*chemical shift*" effect — the change of proton resonant frequency – of hydrogen (^{1}H) which is present in almost all organic compounds. This term was developed by N. Ràmsey in 1951, for defining a distinction between frequencies of separate spectral peaks(38). This effect causes the resonant frequency of the NMR signal to change by small amounts (usually expressed in terms of *parts per million* (ppm) of the resonant frequency), because the local magnetic field surrounding each nucleus depends on both the structure of its surrounding electrons (i.e. the chemical structure of the molecule that the nuclei occur in) and also on the magnetic properties of neighbouring nuclei. Thus, nuclei in different chemical environments will exhibit different resonant frequencies (or spectra in the case of molecules with multiple different nuclei), and NMR spectra can thereby be used to identify both the structure and relative concentrations of the molecules within the sample(39).

Proton spectroscopy just requires additional software coupled with an existing MRI scanner, and only requires an additional 10-15 minutes of imaging time per patient. The spectra generated can be used to

monitor metabolic changes in tumours, infections, degenerative disease and even stroke. The MR spectra need to be interpreted together with the MRI images in order to arrive at a diagnosis(40).

2.2.1. Basic Physical Principles

Protons inherently resonate between 10 MHz and 300 MHz at 0.3T and 7T magnetic field strengths respectively. Higher field strengths provide better separation of metabolite peaks due to higher signal-to-noise ratio. At 1.5T metabolites resonate at between 63-64 MHz.

As mentioned above, MRS is based on the phenomenon of chemical shift. This frequency shift is measured relative to a known standard, which in the case of proton MRS, is tetramethylsilane (TMS), an organosilicon compound. TMS is the accepted standard for calibrating chemical shift in nuclear magnetic resonance in organic solvents. Rather than expressing this chemical shift in absolute frequencies (which varies with magnetic field strength), the resonance is expressed as a very small ratio (parts per million - ppm). This ratio is to the standard TMS whose resonance is at 0 ppm. Using this ppm ratio enables spectra to be reproduced at different field strengths. The ^{1}H protons being analysed are part of methyl groups in the organic compounds of interest. The fact that the chemical environments of these protons differ with each different metabolite, enables different metabolites to be identified on a spectrum, and also allows the spectra to be reproduced(37). The ratio between metabolites peaks in a spectrum, decrease or increase of the height of separate peaks in a spectrum, are like fingerprints of brain biochemistry: on their basis, it is possible to make a non-invasive assessment of the biochemical process in tissues(38).

In conventional MR imaging the signals from all protons in the volume of interest are used to create an image. This would be counterproductive in MRS because the signals from fat and water would be so huge (50,000 times greater), as to make the peaks of the metabolites of interest - invisible(37). The fat and water are hence eliminated. Fat is avoided by appropriate placement of the voxel away from tissues which are known to contain fat, like the bone marrow and scalp. The suppression of water is achieved by specialized software algorithms and sequences such as Chemical-Shift Selective (CHESS) and Inversion Recovery technique(40). Single voxel spectroscopy utilises one of two pulse sequences, either STEAM or PRESS, together with specialized software utilising Fourier transform, to separate the received signal into individual frequencies. Different molecules are thus separated because the protons in each metabolite will resonate at different frequencies because of the different effects on the magnetic field of the protons by the surrounding electron cloud in different molecules.

STEAM (Stimulated Echo Acquisition Mode) is similar to gradient echo in that it uses a 90° refocusing pulse to receive the signal(40). STEAM provides higher resolution spectra, but is easily distorted by patient motion, and has lower signal-to-noise(38). PRESS (Point Resolved Spectroscopy) is similar to spin echo in that it uses a 180° refocusing pulse(40). PRESS produces slightly lower resolution spectra, but is more resilient to patient motion, and provides better signal to noise(38). PRESS is the most commonly used technique for ^{1}H-MRS.

Multi-voxel MRS is also known as Chemical Shift Imaging (CSI). Spectroscopic Imaging (SI) utilises the CSI data and overlays it on an image. The brightness or colour intensity represents the concentration of the metabolite in that area of the image.

2.2.2. Technical Considerations

^{1}H-MRS can be performed as a single voxel (1D) (**Figure 2**), single slice - multi-voxel (2D) (**Figure 3**), or multi-slice- multi-voxel (3D) technique, depending on available software. The normal technique requires identifying the voxel (volume of interest) from a standard MRI performed immediately at the start of the examination. This standard MRI can either be a T2W, FLAIR or gadolinium enhanced T1 weighted image. Usually but not always, a voxel from the normal corresponding contralateral side is also analysed as a control, especially in single-voxel MRS.

Just like conventional MRI, MRS is also affected by factors such as field strength, TE, TR and size of the voxel. Higher field strengths, like in new 3T scanners, provide better spectra than 1.5T.

In the same way that altering the TE changes the signal intensities of different tissues in MR imaging, in MRS the TE will determine which metabolite peaks are best seen in the spectrum. Usually a short TE of 30-35 msec, and a long TE of 270-288 msec is used to demonstrate the common metabolites N-acetyl aspartate (NAA), creatine (Cr), and choline (Cho) – (due to their long T2 relaxation times). However, since Lipid (Lip) and Lactate (Lac) occur at the same position at these TE's, an intermediate TE of 135-144 msec is then required to separate them, because it inverts the lactate peak.

2.2.3. Principal Metabolites Measured in MRS

The metabolites which are commonly encountered in MRS of the brain are listed in (**Table 1**). The specific ppm at which their peaks appear is used to identify them. Each represents an important metabolic and biological marker. 0 ppm, which represents the reference standard TMS, is displayed at the right edge of the spectrum. Chemical shift increase in ppm is portrayed towards the left (**Figure 2**) (48).

While no less than 200 metabolites are generated in the brain, we are blinded to most. This is most likely because either they occur in very minute concentrations, or that MRS cannot decipher these molecules. These include substances like DNA, many enzymes and important neurotransmitters like dopamine and acetylcholine. However, proton MRS however employs mainly seven metabolites which are metabolically important and easily detectable. These principal metabolites and what they represent include:

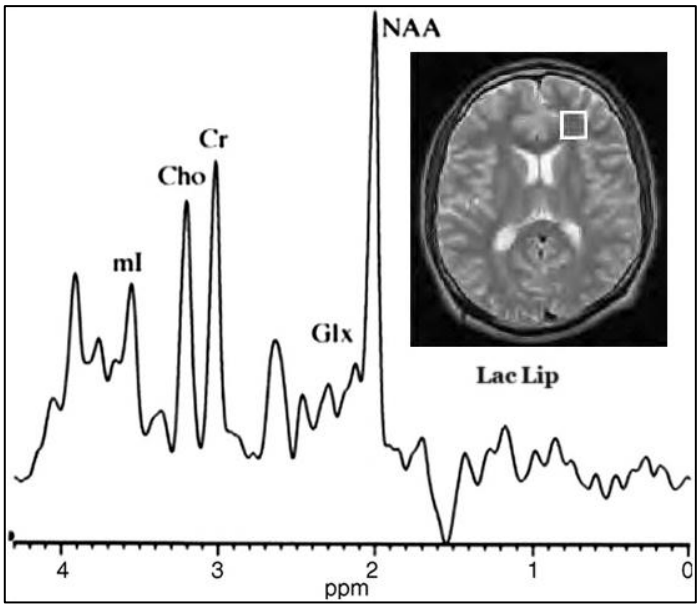


Figure 2: Single-voxel proton MRS of brain tissue in a normal volunteer. The peaks of main metabolites are marked on the image (38, 41).

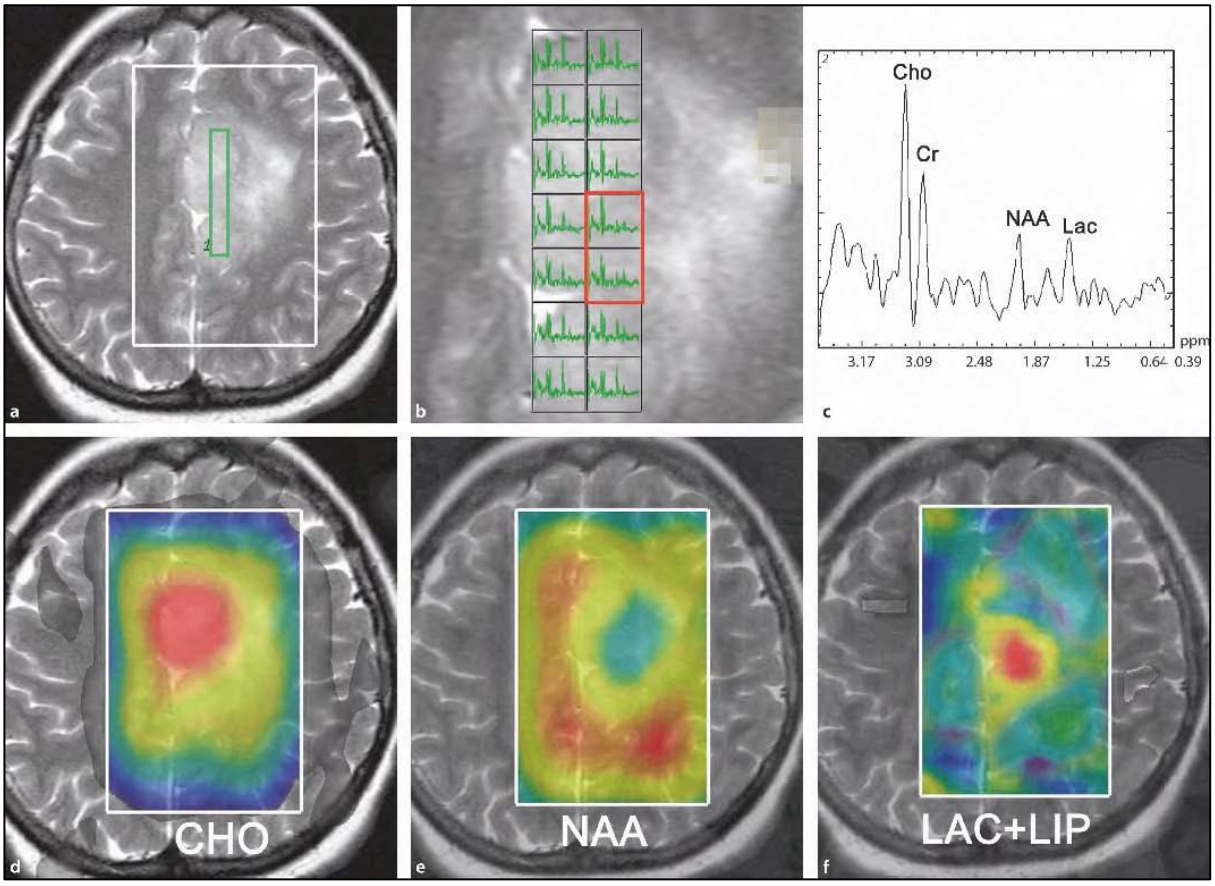


Figure 3: Multi-voxel proton MRS in a patient with a brain tumour. **a.** Spectra presentation in each voxel; **b.** enlarged image with measured points placement; **c.** spectrum of tumour's tissue, with typical glioma metabolite changes; **d–f.** colour map of different metabolite contents. ©*Diagnostic Neuroradiology, Kornienko et al. Springer 2009*

Table 1: Observable proton metabolites in MRS (40).

Ppm	Metabolite	Properties
0.9 - 1.4	Lipids (Lip)	Products of brain destruction
1.3	Lactate (Lac)	Product of anaerobic glycolysis
2.0	N-acetyl aspartate (NAA)	Neuronal marker
2.2 - 2.4	Glutamine / GABA (Glx)	Neurotransmitters
3.0	Creatine (Cr)	Energy metabolism
3.2	Choline (Cho)	Cell membrane marker
3.5	*myo*-Inositol (mI)	Glial cell marker
Other Observable Metabolites		
1.2	Ethanol	Triplet
1.48	Alanine	Present in Meningiomas
3.4 & 3.8	Glucose	Increased in Diabetes
3.8 Mannitol	Rx for increased ICP	

N-acetyl aspartate (NAA)

- Resonates at 2.0 ppm chemical shift

NAA is a by-product of amino acid metabolism found in viable neurons and axons. It therefore serves as a very definitive marker of viable neurons(42). Grossman and Yousem say "everything except Canavan has low NAA, high Choline"(43). When brain matter is destroyed, be it white or grey matter, NAA is reduced(44),(37). This can be seen in brain tumours and white matter diseases(45). An increase in NAA is only seen in Canavan disease (**Figure 4**)(46).

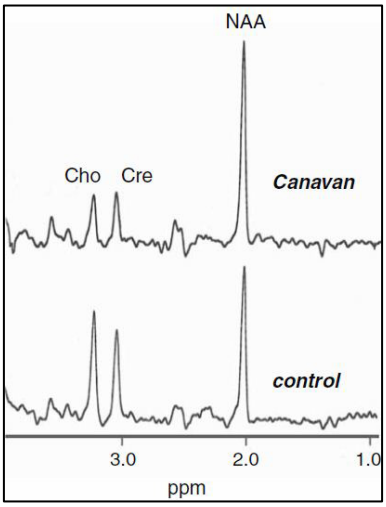


Figure 4: MR spectra in a patient with Canavan disease compared to a normal control. In Canavan NAA is markedly increased with decline in Creatine and Choline (45).

Creatine (Cr)

- Resonates at 3.0 ppm chemical shift

It is a marker of energy metabolism and is found in all active tissues. It is maintained at a constant level, even in disease, and is hence used as a reference to compare other peaks with(44). Thus when interpreting other metabolite peaks, rather than changes in individual heights, their ratios to Creatine is what is more important(37). This metabolite/Cr ratio is usually reproducible.

Choline (Cho)

- Resonates at 3.2 ppm chemical shift

In MR spectroscopy choline is a marker of cell membrane and myelin turnover. It is elevated in conditions of rapid cell turnover, as in tumours; and myelin loss, as in demyelinating diseases and gliosis. The more aggressive a tumour, the higher the choline(44). It is also used to distinguish high grade gliomas from abscesses and metastases, as only gliomas will show elevated choline in the peri-tumoural non-enhancing region(37).

Lactate (Lac)

- Resonates at 1.3 ppm chemical shift.

It is a product of anaerobic glycolysis, and so is only seen in necrotic tissues, not in normal tissue. It is also seen in very aggressive tumours where oxygen supply cannot keep up with demand. It is also seen in infections and acute stroke(44).

It is identified by its double peak at long TE. It may overlie the lipid peak, so an intermediate TE spectrum is obtained in which the lactate peak is inverted (**Figure 5**).

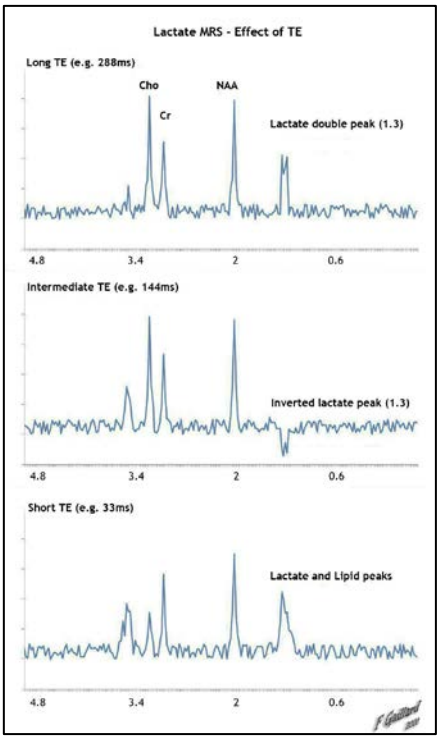


Figure 5: Lactate peak on MRS varies according to TE (44).

Lipids (Lip)

- Resonates at 0.9 - 1.3 ppm chemical shift.

It is not seen in normal MRS unless the study voxel includes bone marrow or scalp fat. It is a marker of cell membrane breakdown, and an indicator of necrosis. It is thus seen in tumour necrosis, radiation necrosis, and abscesses(37).

Glutamine / GABA / Glutamate complex (Glx)

- Resonates at 2.2 - 2.4 ppm chemical shift

These are amino acids and neurotransmitters, and are seen in excess in gliomas(44). Although glutamate and glutamine cannot be distinguished at 1.5T (hence collectively termed Glx peak), they can at 3.0T(37). Glutamate is also seen in hypoxic brain after stroke and neonatal hypoxia(41), and in HIV and MS(47).

Myo-inositol (mI)

- Resonates at 3.5 ppm chemical shift

Myo-inositol is a marker of gliosis. It is also increased in some glial tumours, Alzheimer's disease and PML (Progressive Multifocal Leukoencephalopathy).

Alanine

- Resonates at 1.48 ppm chemical shift

It is primarily seen in meningiomas and some abscesses(39).

2.3. Interpretation of MRS Spectra

In interpreting an MR spectrum, one must first identify the metabolites by their chemical shift. This is determined by their location on the ppm baseline at which they occur. This is read from the extreme right (0 ppm) going to the left. The scale being in ppm is identical, regardless of which sequence, magnet strength or MRI scanner brand is used. The heights of the individual peaks reflect each metabolite's concentration.

All peaks are identified and recorded. The common peaks are identified from right to left: lipids (Lip) at 0.9 ppm, lactate (Lac) at 1.3 ppm, N-acetyl aspartate (NAA) at 2.0 ppm, glutamate/glutamine (Glx) at 2.2 ppm, creatine (Cr) at 3.0 ppm, choline (Cho) at 3.2 ppm, and myo-inositol (mI) at 3.5 ppm(42) (**Figure 6**).

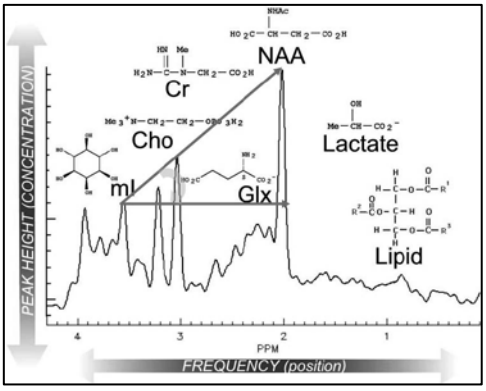


Figure 6: Normal brain MRS spectrum. Hunter's angle is also demonstrated (42).

An additional secondary creatine peak may be seen at 3.9 ppm, but there should be nothing beyond that. If any peak is seen beyond 4.7 ppm or the baseline is seen sloping upwards, it means that water suppression was not achieved by the acquisition sequence(37) (**Figure 7**).

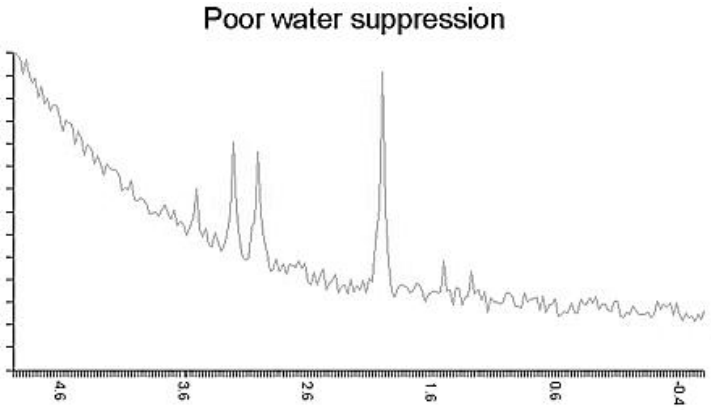


Figure 7: Effect of poor water suppression on MRS (44).

Apart from the individual peaks, it is important to look at metabolite ratios, namely NAA/Cr, NAA/Cho, Cho/NAA and Cho/Cr. Normal and abnormal values are shown in (**Table 2**) for quick reference. Similar data from different studies and different MR machines is summarized in (**Table 3**).

Table 2: MRS Metabolite Ratios (40).

Metabolite Ratio	Normal	Abnormal
NAA/Cr	2.0	< 1.6
NAA/Cho	1.6	< 1.2
Cho/Cr	1.2	> 1.5

The graphical appearance of spectra may differ from machine to machine, or with different magnet strength and pulse sequence. However, the chemical shifts of the metabolites will always correspond. Modern software usually identifies the common peaks. For purposes of metabolite quantification, rather than the height ("amplitude") of the peak, the "integral" is used, which represents the peak area. There are many other metabolites which may be identified on MR spectra, but are not commonly seen. Each has an identifiable chemical shift. Therefore if an abnormal peak is seen, it most often has diagnostic significance. These are presented in **Table 22**, Appendix B(41).

Table 3: MRS Normative Data in Regional Metabolic Ratios (41).

Posterior Cingulate Gyrus Grey Matter					
MR system	**N**	**Age**	**NAA/Cr**	**Cho/Cr**	**mI/Cr**
General electric	13	26.0 ± 7.0	1.63 ± 5.5%	0.65 ± 13.8%	0.52 ± 9.6%
Siemens	8	21.3 ± 2.4	1.68 ± 16.2%	0.65 ± 9.8%	0.57 ± 33.4%
Philips	8	21.8 ± 2.6	1.63 ± 7.5%	0.52 ± 9.4%	0.40 ± 14.9%
Mean grey matter	**29**	**23.5 ± 5.4**	**1.65 ± 9.8%**	**0.61 ± 13.6%**	**0.49 ± 24.1%**
Left Parietal White Matter					
General electric	13	26.0 ± 7	1.98 ± 7.6%	0.96 ± 12.5%	0.59 ± 13.6%
Siemens	8	21.7 ± 2.7	2.04 ± 14.7%	1.09 ± 10.1%	0.57 ± 27.9%
Philips	8	22.9 ± 2.1	1.88 ± 5.9%	0.91 ± 13.2%	0.48 ± 18.8%
Mean white matter	**29**	**23.5 ± 5.4**	**1.97 ± 9.2%**	**0.97 ± 13.3%**	**0.54 ± 20.8%**
All spectra were acquired on clinical MRI scanners using the manufacturer's automated MRS technique (PRESS TE 35 ms, TR 1500 ms). Values given are mean with standard deviation.					

Traditionally, two spectra were obtained; one from the area in question, and one from a control. There would need to be a difference in height of at least 20% for it to be classified as disease. However, nowadays with modern software, the process is simplified and diagnostic sensitivity improved (detect 10% difference) by comparing ratios (Cho/Cr, NAA/Cr etc.) to tables of normal data particular to the scanner and the TE used. This does away with the need for a control spectrum.

However the importance of controls has also been documented. Spectroscopy scans of focal brain lesions are often much easier to interpret if spectra from presumed normal brain in the contralateral hemisphere are available for comparison(39). Finally, the interpretation of spectra from very young children (term and preterm neonates, and children less than 4 years of age) are particularly challenging because of the rapid changes in brain metabolism that occur in these age ranges(39). (**Figure 8**) and (**Figure 9**) below show regional and age related variations in spectra that may pose a challenge during interpretation.

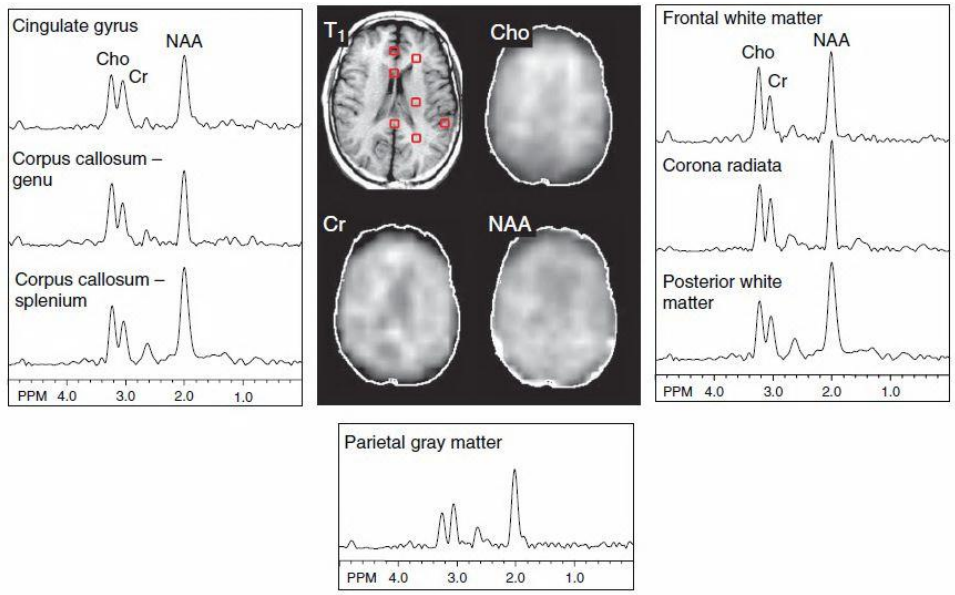


Figure 8: Normal volunteer, 49 years old. Metabolic images show appreciable regional variations (39).

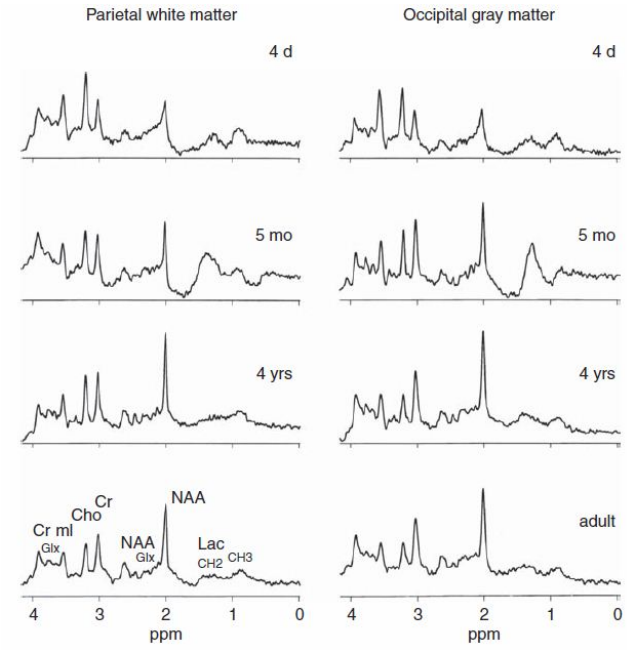


Figure 9: Age-related variations in MRS – the normal developing brain. As the brain develops, NAA increases and Cho and mI decrease so that by about 4 years of age (in these locations) the spectra are essentially indistinguishable from those in young adults (39).

2.4. Spectra in specific conditions

2.4.1. Brain Tumours

MRS can be used to propose the degree of malignancy. NAA decreases as neurons are destroyed. Aggressive tumours have high metabolism thereby rapidly depleting their energy stores, which results in a reduced creatine. Choline reflects cell membrane turnover, and so hypercellular tumours with fast growth rates will result in elevated choline. The presence of lactate implies anaerobic glycolysis, which is seen when tumours outgrow their oxygen supply. Lipid is elevated in areas of necrosis where there is cell

membrane breakdown. However, for accurate MRS assessment, the voxel needs to be carefully placed; preferably over areas which enhance with contrast(40).

Choline is elevated in all tumour types due to altered membrane metabolism, and shows correlation with cellular density and indices of cell wall proliferation. N-acetyl-aspartate (NAA) decreases with tumour infiltration and substitution of normal neural and glial cells(39).

- The Cho/NAA ratio is, therefore, a useful parameter particularly in most adult and paediatric primary brain tumours, with a higher ratio correlating with higher cell density and generally associated with a poor prognosis(39).
- Increasing Cho/NAA and Cho/Cr ratios in serial exams of a primary astrocytoma are suggestive of transformation to a higher grade (**Figure 10**, **Figure 11**).
- By following metabolic changes, ^{1}H-MRS can be useful in monitoring disease progression or response to therapy.
- ^{1}H-MRS studies can also be particularly useful in distinguishing neoplastic from non-neoplastic lesions, and differentiating recurrent tumour from predominantly delayed radiation necrosis(39).
- MRS cannot always distinguish between primary and secondary tumours of the brain.

Glioma

- As tumours progress to higher grade :
  - NAA - ↓
  - Cr - ↓
  - Cho - ↑, **and in margins of enhancement**
  - Lip - ↑
  - Lac - ↑

Non-glial tumours

- NAA - ↓ or **absent**
- Cho – **not seen in margins of enhancement**.

- Analysis of MRS data showed significantly higher Cho/Cr ratios in high-grade than in low-grade tumours (**Figure 10**, **Figure 11**). A Cho/Cr ratio cut-off value of 2.33 had the highest accuracy in identification of high-grade tumours(48).

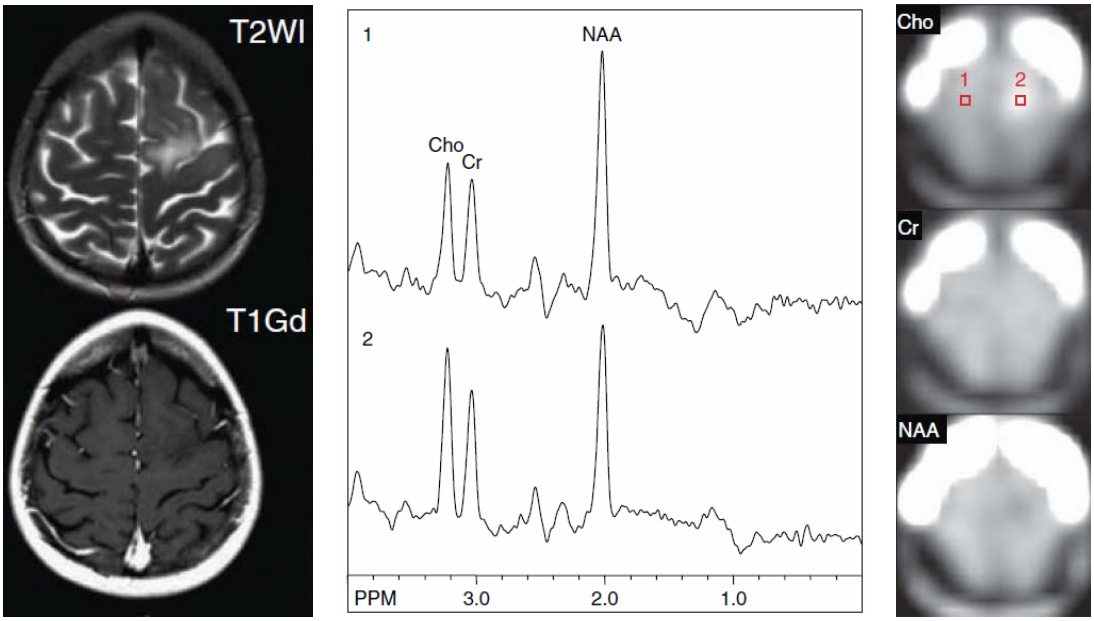


Figure 10: Low-grade astrocytoma. Note mild Cho increase and moderate NAA decrease in the tumour (2) compared with the contralateral normal spectrum (1) (39).

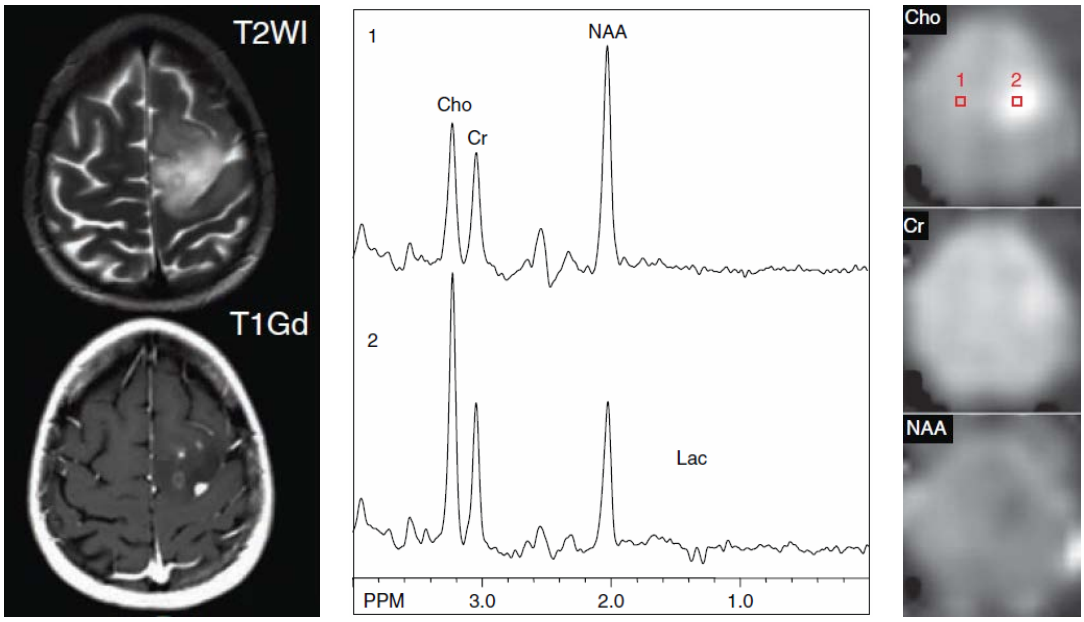


Figure 11: At follow-up seven months later. Note that Cho has significantly increased and NAA decreased in the tumour (2) compared with the contralateral normal spectrum (1). Note also appearance of a small lactate peak in the spectrum from the tumour. High-grade anaplastic astrocytoma diagnosed after surgery (39).

2.4.2. Brain Abscesses

- Pyogenic abscess have a unique metabolic pattern with decreased levels of all normally observed brain metabolites, and elevation of succinate, alanine, acetate, and amino acids, as well as lipids and lactate (**Figure 13**). This pattern is quite distinct from that seen in brain tumours(39).
- Tuberculomas are characterized by elevated lipid and an absence of all other resonances (**Figure 13**).

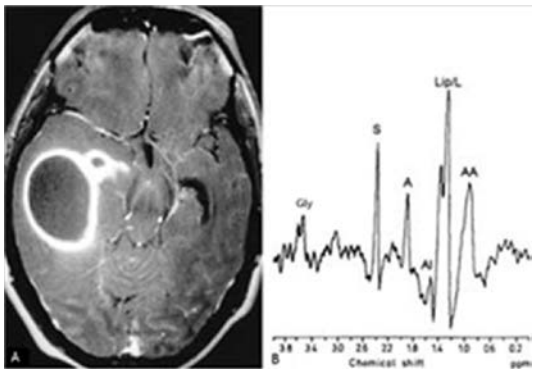


Figure 13: Pyogenic abscess. MRS shows multiple amino acid peaks; glycine (3.6ppm), succinate (2.4ppm), acetate (1.9ppm), alanine (1.5ppm) and valine (0.9ppm). Lipid/Lactate is also elevated (1.3ppm) (29).

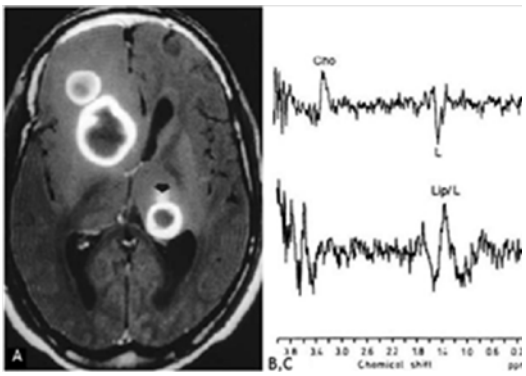


Figure 13: Tuberculoma. No amino acids seen. Only elevated Lipid/Lactate seen at 1.3ppm (29).

- Toxoplasmosis shows prominent peaks from lactate and lipids(44). It looks similar to lymphoma, but MRS makes the separation; lymphomas have elevated choline, and toxoplasmosis does not(40).

- In 2010, Khanna et al presented a case of an unusual mass-like "giant" extra-axial tuberculoma during pregnancy. On conventional imaging alone, it looked like a meningioma. However, MR spectroscopy showed typical findings of tuberculoma; and the diagnosis was confirmed (**Figure 14**)(49).

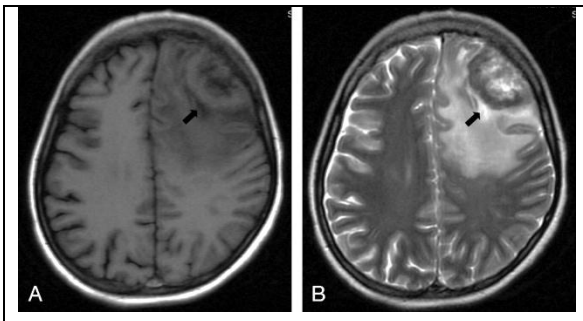


Figure 14a: Lesion in left frontal region (A) T1w - hypointense with isointense rim, (B) T2w - hypointense with central hyperintensity. Displacement of surrounding brain with CSF cleft. Significant surrounding oedema (49).

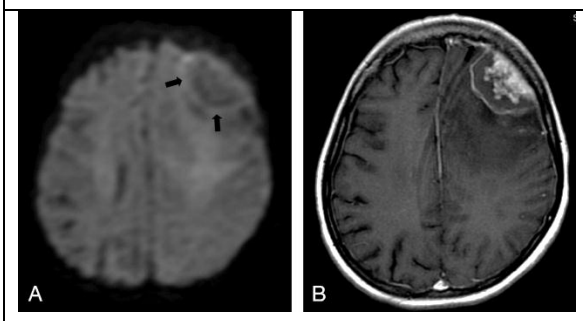


Figure 14b: (A) DWI - lesion is hypointense with surrounding hyperintense rim. (B) T1+Gd - Central enhancement, with rim enhancement beyond a thick non-enhancing component. Enhancing dural tail also seen (49).

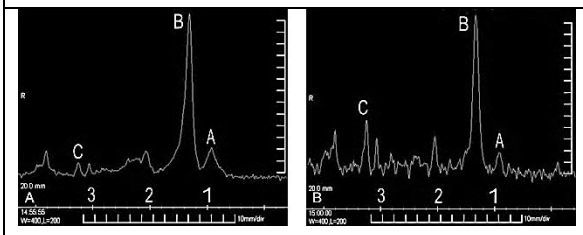


Figure 14c: At MRS prominent Lipid/Lactate peaks seen (0.9 and 1.3ppm). Markedly reduced NAA, Creatine and small Choline peak at 3.2ppm - typical of tuberculoma (49).

2.4.3. Is it a GBM, metastasis, or an abscess?

The differential diagnosis of a ring enhancing mass is a common question encountered by radiologists. Multi-voxel MRS best demonstrates elevation of Cho in the enhancing rim and in the peri-lesional T2 hyperintensity. Single-voxel spectroscopy can also be done in these regions. If Cho is elevated in both areas, a likely diagnosis of GBM (Glioblastoma Multiforme) may be suggested (**Figure 15**). Elevation of Cho in the enhancing rim but not in the surrounding tissue suggests the diagnosis of metastasis. In spectra derived from the necrotic/cystic core of the mass, accumulation of lipids or lactate without elevated Cho is not a specific finding; as this can also be seen in abscesses; thus, the acquisition of an additional spectrum with short TE showing other amino acid peaks would confirm the diagnosis of a pyogenic abscess(39).

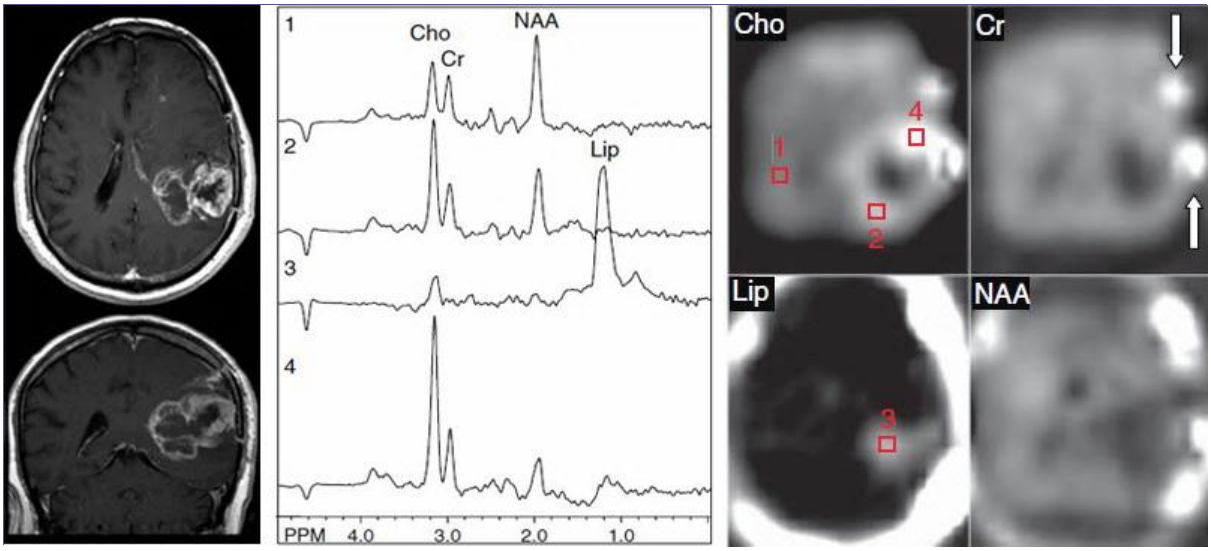


Figure 15: Recurrent GBM. Surgeon wants to rule out abscess. A normal spectrum from the contralateral temporal lobe (1) is shown for comparison. Note the very high Cho/ NAA in two non-enhancing areas of the mass (2, 4), a likely sign of high cellular density and proliferation without necrosis. In the deeper seeded enhancing component of the mass (3) there is a strong signal from mobile lipids, a sign of necrosis, in association with depletion of Cr and NAA; the Cho signal is also relatively weak (39).

2.4.4. Radiation effects

Radiation changes looks similar to tumour; especially in patients who have had radiotherapy, it may appear as tumour recurrence. The presence of increased Choline suggests tumour recurrence. In radiation changes, NAA, Cr and Cho are all reduced. In radiation necrosis, lipid/lactate is also seen(37).

Radiation Effects
• ↓NAA, ↓Cr, ↓**Cho**, (↑Lip, ↑Lac may be present)
Tumour Recurrence
• ↑**Cho**

2.4.5. Cerebral Ischemia and Infarction

- Although the diagnosis of cerebral infarct is often made based on clinical information and diffusion weighted MR imaging, in a few instances, an infarct may appear as a mass suspicious of a tumour (**Figure 16**).
- In ischemia, the brain crosses over to anaerobic glycolysis, and as a result, lactate builds up. This is reflected on MRS as an elevated lactate peak. Choline increases, and NAA and Creatine peaks are decreased. If the brain tissue becomes infarcted, Lipid peak will be seen(39, 40).

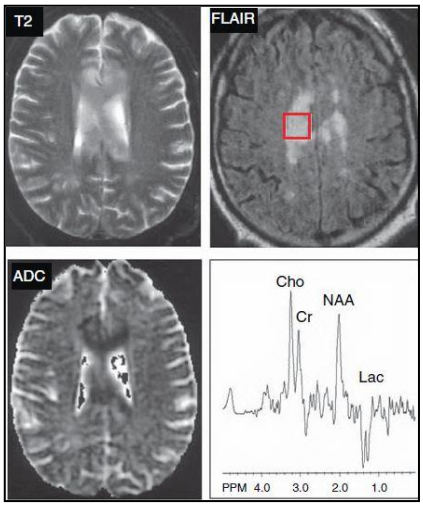


Figure 16: T2 and FLAIR MRI show a lobular hyperintense signal abnormality with expansion of the corpus callosum, considered suspicious for neoplasm. ADC maps show reduced diffusion and MRS shows an elevated lactate and reduced NAA, consistent with acute ischemic disease. (39)

3. Problem Statement

Despite advances in conventional MR imaging, in about 10-30% of patients with intracranial mass lesions, the differentiation between cerebral infarcts, metastases, high and low grade tumours; and discrimination between neoplastic and non-neoplastic lesions like abscesses is ambiguous (1, 2, 6). This is because often such lesions appear similar on conventional imaging. Often the alternative and only way to reach a diagnosis is by open biopsy, which is costly and invasive, and associated with its own morbidity(2).

The burden of neurosurgical diseases in developing countries, including Kenya, is immense. The effect is felt on individual patients, society and the economy. The number of trained specialist physicians and surgeons in this field are simply not enough. According to Kahamba et al, in East Africa, there is currently "one neurosurgeon per 9 million people", whereas in the United States the ratio is 1:62,500(50). According to Qureshi and Oluoch-Olunya, more than 90% of patients with neurosurgical diseases in Kenya end up at either Kenyatta National Hospital, or Moi Teaching and Referral Hospital. Although there are a handful of well-trained neurosurgeons; they are often overwhelmed. The policy of cost-sharing by patients means that the quality of care is often sub-optimal, as most patients cannot afford the best care(51).

Some of the most common neurologic manifestations of HIV infection are intracranial mass lesions. These are most commonly abscesses, tumours or sometimes vascular lesions(52).

Proton MRS can provide a relatively affordable non-invasive alternative to solving the diagnostic dilemma in the 10-30% of situations where conventional MRI is inadequate; and hence expediting treatment, be it surgery, pharmacotherapy or radiotherapy.

4. Study Justification

Despite advances in conventional MR imaging, in about 10-30% of patients with intracranial mass lesions, their diagnosis is unclear. No local data is available regarding the incidence of intracranial mass lesions, and the only data pertaining to developing countries comes from studies in the Indian subcontinent, a few studies from southern Africa and Nigeria, and studies on African and Asian immigrants conducted in the west(53), (54).

Conventional MRI is available at the Kenyatta National Hospital, and a few of the major private diagnostic imaging establishments in Nairobi and Mombasa. While conventional MRI will answer the diagnostic question in 70-90% of patients with intracranial lesions, there remain 10-30% of conditions in which conventional MRI, despite its exceptional depiction of soft tissue characteristics, cannot answer that question(1). This is because often such lesions appear similar on conventional imaging. Often the alternative and only way to reach a diagnosis is by open biopsy, which is costly and invasive(2). With the current burden of neurosurgical disease in this part of the world being high(50), proton MRS can provide a relatively affordable non-invasive alternative to solving the diagnostic dilemma in these situations, and if possible obviate the need for open biopsies in some cases.

Today the large majority of MRI providers do not provide MRS facilities, and on top of that only a handful of clinicians request MRS examinations, because of the scarcity of service providers and the lack of awareness among clinicians on the diagnostic benefit that MRS can provide. The author hopes that the outcome of this study may enlighten other clinicians and diagnosticians on the utility of MRS as an adjunct to MRI.

5. Research Question

What role does ^{1}H-MRS play in improving the imaging diagnosis of intracranial mass lesions in our local setup?

6. Hypothesis

MR spectroscopy added to MRI improves the imaging diagnosis of intracranial mass lesions in our local setup.

7. Study Objectives

7.1. Broad Objective

The main objective of this study was to evaluate the clinical utility and diagnostic value of ^{1}H-MRS added to MRI for the diagnosis of intracranial mass lesions in our local setup.

7.2. Specific Objectives

- To determine the indications for which MRS is requested in this country.
- To provide data on MRS parameters and patterns of different intracranial mass lesions encountered locally.
- To determine the role of ^{1}H-MRS in differentiation between neoplastic and non-neoplastic lesions.
- To determine the role of ^{1}H-MRS in differentiation of low- and high-grade tumours.

8. Study Design and Methodology

8.1. Study Duration and Location

This is a retrospective study looking at brain MR spectroscopy examinations done over a period of 12 months from September 2012 to September 2013 that were conducted at Plaza Imaging Solutions, an outpatient radiology practice located in Nairobi.

8.2. Sampling

All patients who present to the imaging centre with a request for brain MRS from their clinician, and who meet the inclusion criteria; were consecutively recruited. As mentioned in Chapter 12 a limitation in this study is that majority of patients recruited in this study may already have had prior imaging with inconclusive diagnosis. Due to cost implications, the decision to refer a patient for MRS is often made by the clinicians, either on their own, or upon recommendation of the radiologist. This, coupled with the fact that a consecutive series sample selection method was used; may have introduced an element of selection bias

Inclusion Criteria

- Patients aged>4years with focal intracranial mass lesions. By age 4 years, MR spectra of the brain are indistinguishable from those of adults(39).
- Patients with MRI data of satisfactory imaging quality for diagnostic purposes.

Exclusion Criteria

- Patients with contraindications to conventional MRI.
- Patients aged <4 years – diagnostic challenges because of the rapid changes in brain metabolism that occur below 4 years age(39).
- Patients with poor quality MRI data, without any diagnostic significance, mostly due to movement artefacts and/or non-compliance on their part.
- Patients with demyelinating diseases and other lesions not categorised as intracranial mass lesions.
- Patients whose MR spectra were of poor diagnostic quality; either due to poor water suppression, contamination by fat or degradation due to magnetic field inhomogeneities from haemorrhage.

There was no discrimination based on ethnic, religious and political background or socio-economic status in this study.

8.3. Sample Size and Considerations

Purposeful consecutive sampling was chosen for this study because currently very few; an average of five patients per month, are referred for brain MRS. Out of a total of 68 brain MRS examinations performed during the study period, 63 patient's MRS examinations satisfied the inclusion/exclusion criteria to be included in the study .

8.4. Sample Size Determination

Sample size calculation was based on % sensitivity and specificity of ^{1}H-MRS – 96% (95% CI, 83%–99%) and 100% (95% CI, 86%–100%) respectively, as reported by Mishra et al(21) and 96% (95% CI, 78%–100%) and 88% (95% CI, 75%–96%), respectively by Astrakas et al(16). The method used to determine sample size was based on tables of number of cases (or controls) for expected sensitivities (or specificities) as described by Flahault et al in an original article on sample size calculation for "design accuracy in diagnostic test studies"(55). Assuming the likely sensitivity of the diagnostic test is $\boldsymbol{\pi}$, and we target for the $\boldsymbol{1-\alpha}$ lower confidence limit for $\boldsymbol{\pi}$ to be greater than $\boldsymbol{\pi-\delta}$ with probability $\boldsymbol{1-\beta}$; the required number of subjects is:

$$\boldsymbol{n = \frac{z1-\beta\sqrt{\pi(1-\pi)} + z1-\alpha\sqrt{(\pi-\delta)(1-\pi+\delta)}}{\delta 2}}$$

Where:

$\boldsymbol{n}$= required sample size.

$\boldsymbol{\pi}$= % sensitivity of ^{1}H-MRS. This is taken as 96%(16),(21)

$\boldsymbol{\delta}$= degree of precision (maximum distance within which the 95% lower confidence limit is required to fall) = 12%. This is estimated from 83% by Mishra et al.(21)

$\boldsymbol{z1-\beta}$= standard normal for 80% power = 0.84

$\boldsymbol{z1-\alpha}$= standard normal for 95% confidence interval = 1.96

When substituted, sample size ($\boldsymbol{n}$) required was **53** patients.

The number of patients included in the study was **63** which was more than required.

8.5. Method

All patients were investigated under a constant single-volume ^{1}H-MRS protocol following structural MRI imaging. All imaging and spectroscopic studies were carried out with a 1.5T Phillips Intera whole-body magnetic resonance scanner using a circularly polarized head coil.

Either T2 weighted, or gadolinium enhanced T1weighted axial images were used for voxel localization. Water-suppressed single-voxel spectra were acquired using a double spin-echo localization technique (PRESS, point-resolved excitation spin-echo sequence) with frequency-selective water suppression.^{1}H-MRS was performed using automated spectroscopy sequences at short TE of 31msec, and intermediate TE of 144msec. A voxel of 2–8 mL, depending on the size of lesion, was placed within the lesion.

All MRI and MRS examinations were reported by the attending radiologists. PACS archived images and reports were used to record and analyse the imaging and spectroscopy findings.

8.6. Study Variables

These included:

1. MRI diagnosis
    a. No single diagnosis – if more than one differential diagnosis was being considered
    b. Single diagnosis – if the radiologist was certain of any diagnosis e.g. High grade glioma, Tuberculoma etc.
2. Indication for MRS Mass lesion spectrum (MRS findings):
    a. Location (ppm) of observed metabolites
    b. Observed spectrum appearance
    c. Metabolite values
    d. Metabolite ratios
3. MRS Diagnosis
    a. No single diagnosis – if more than one differential diagnosis was being considered
    b. Single diagnosis – if the radiologist was certain of any diagnosis e.g. High grade glioma, Tuberculoma etc.
4. Did MRS improve the imaging diagnosis? Yes or No. 'Improvement' was based on whether or not a single imaging diagnosis was obtainable after MRS in a patient where using MRI alone it was not.

8.7. Data Management and Analysis

A structured questionnaire was used to collect the data. The MRI and MRI+MRS findings were reviewed independently. The diagnosis made by interpreting MRI alone was tabulated against diagnosis made by interpreting MRI and MRS together.

MR Spectra were obtained at short TE of 31msec and intermediate TE of 144 msec of the lesions under interrogation. Metabolite signals located at 0.9, 1.3, 2.0, 3.0, 3.2 and 3.6 ppm, which were attributed to lipids (Lip), lactate (Lac), N-acetyl-aspartate (NAA), creatine (Cr) and choline (Cho) and myo-inositol (mI) were obtained. Metabolite peaks heights were determined by observing the spectral plots, and determining whether they were unchanged, increased, or decreased. Choline is better elaborated at TE 144 msec, and was read at 144 msec. Lipid is better elaborated at TE 31 msec, and thus read at TE 31msec at 0.9 and 1.3ppm. Lactate elevation was recorded if an inverted doublet was seen at 1.3ppm at TE 144msec. Myo-inositol is elaborated at TE 31 msec, and was thus read at TE 31 msec.

The second way in which data was quantified was by determining the metabolite ratios of peak height. NAA/Cr, Cho/Cr, NAA/Cho and Cho/NAA ratios were determined. The automated spectral analysis software being utilised relied on the Creatine (Cr) of the interrogated voxel as reference and as such no contralateral reference spectra were required.

If the radiologist was certain of any diagnosis e.g. High grade glioma, Tuberculoma etc., they were recorded as such. If more than one differential diagnosis was being considered, this was recorded as "No single diagnosis". This was done for both MRI and MRI+MRS.

Data was further categorized according to the indication for the MRS examination into clinical subgroups. These were:

- Differentiation of low from high grade tumours.
- Differentiation of non-contrast enhancing lesions such as low-grade gliomas from cerebral infarcts.
- Differentiating contrast enhancing cystic or necrotic brain tumours from abscesses.
- Differentiation of radiation necrosis from recurrent tumour.

The data was coded and entered into a Microsoft© Excel© spread sheet. The data entry forms had quality control checks in order to ensure the accuracy of the data. No patients' personal data was recorded apart from age and gender, so as to determine patient demographic data. The database was encrypted and password protected to ensure secured access.

All data was then cleaned and exported for statistical analysis. Statistical Package for Social Sciences (SPSS© v17.0) was used for the analysis. MRI and MRI+MRS diagnoses, indication for MRS, and MRS findings were summarized and presented using percentages. Then, diagnostic value of ^{1}H-MRS added to MRI was determined.

MRI versus MRI+MRS diagnoses was compared using Wilcoxon Rank test. Chi square test of trend was used to test if MRS improved the diagnosis based on the MRS indications. Also, Chi square test of trend was used to test whether there was significant increase or decrease of the MRS metabolites according to the type of tumour. The mean differences of the MRS parameters across the type of tumours were compared using ANOVA test. Paired T-test was used to compare metabolite changes and ratios between major pairs of diagnoses. All statistical tests were interpreted as significant if P value was ≤ 0.05.

Selected MRI/MRS images of some of the cases seen from the study are presented.

8.8. Fate of Raw Data

During the study, data collection forms and written/printed materials related to the study were always locked securely, so that only the investigator had access to them. All DICOM and JPEG images of participant's MRI scans were stored on encrypted disks, and locked securely so that only the investigator had access to the images. The raw DICOM images were anonymised using the PACS software, and participant images were identified only by their numbers.

Upon completion of the study, all raw data, including data collection forms / questionnaires and any written/printed material, were destroyed by shredding and burning. All DICOM source images and JPEG images of participants' MRI scans were deleted using industry standard deletion and disk wiping software so that it cannot be retrieved.

9. Ethical Considerations

This study employed the use of electromagnetic non-ionizing radiation which has been confirmed to have no hazardous biological effects, on patients in whom MRI is not contraindicated, at the magnetic field strengths typical of present diagnostic equipment.

The following ethical guidelines were used in line with the World Medical Association (WMA) Declaration of Helsinki(56).

- The name, religion and racial background of the patient werenot documented in this study. Patients were identified only by MRI number and alphanumeric patient identity codes to safeguard confidentiality.

- All patients who underwent MRI/MRS examinations were carefully screened for safety. They were informed on the benefits, risks and possible adverse effects of MRI and Gadolinium contrast agent. They were all required at read and understand, and fill-in and sign MRI safety questionnaires and informed consent forms prior to having their MRI/MRS performed.

- No additional imaging examination was done other than the one requested by the referring clinician.

- The patients did not incur any additional cost by participating in the study.

- No blood sample or biopsy specimen was collected as it was not indicated in this study.

- All patients had been managed at the optimal standards as personnel and facilities allowed.

10. Results

A total of 68 patients presented to Plaza Imaging Solutions for Brain MR Spectroscopy between September 2012 and September 2013. Of these, 63 patients satisfied the study's selection criteria and were thus recruited for the study.

Brain MRI and MRS examinations were performed as described in the methodology, and their findings were analysed.

The analysis of the basic characteristics, MRI and MRS findings of these patients is presented below:

10.1. Age and Gender

The average age (Mean (± SD)) of patients with intracranial masses was 45.33 years (±20.14). The youngest patient was 5 years of age while the oldest was 90 years old. The median of patients' ages was 43 years with an interquartile range (IQR) of 31-62.

Of the 63 patients, 32 (50.8%) were male and 31 (49.2%) were female, representing an almost equal 1:1 male to female ratio.

Figure 17 and **Figure 18** present the age and gender distribution of patients with intracranial mass lesions presenting for Brain MR Spectroscopy.

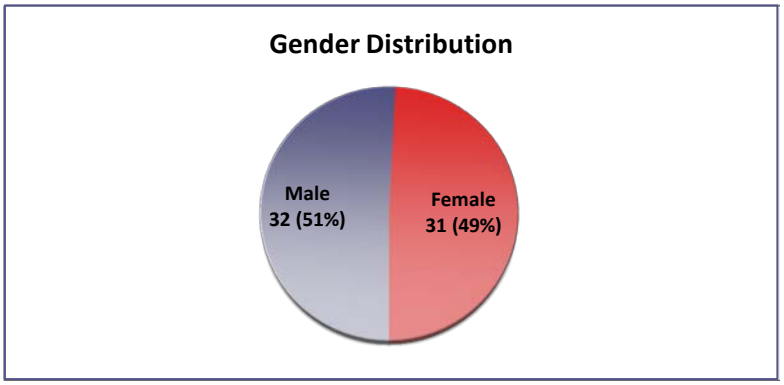


Figure 17: Gender distribution of sample

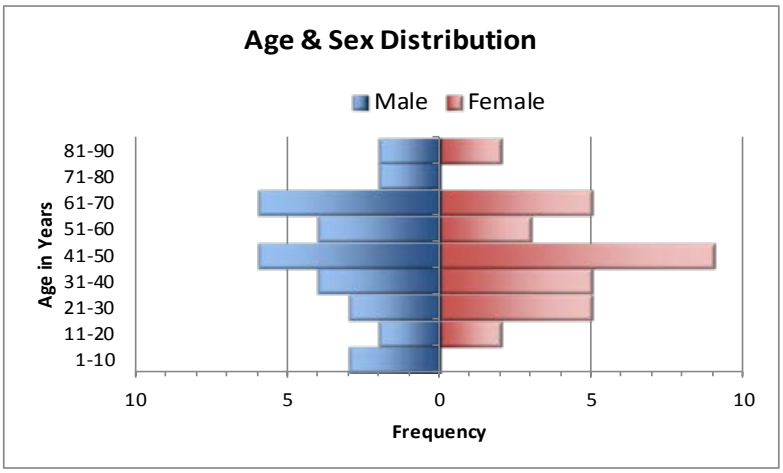


Figure 18: Age and Sex distribution of sample

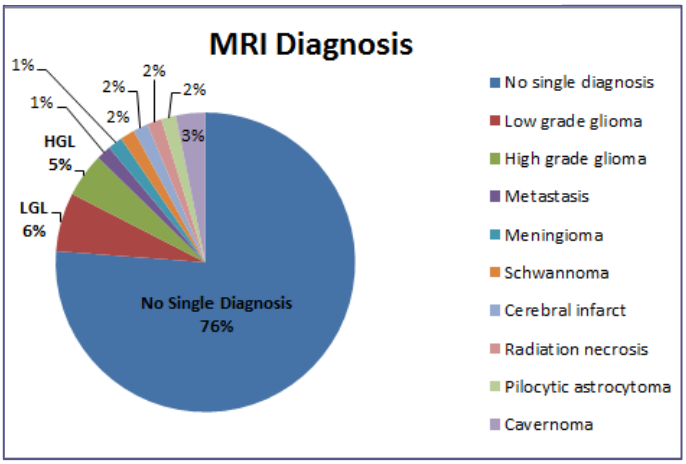
10.2. MRI Diagnosis

All patients referred for brain MRS underwent conventional brain MRI scans before their spectroscopy examinations. All MRI and MRS studies were reported and reviewed with a consultant specialist neuroradiologist who has more than 10 years practice experience in neuroradiology based on clinical information and imaging features. These MRI examinations were initially reported independently of their corresponding MRS findings. When the reporting radiologist had more than one differential, even if there were two indisputable differentials, this was recorded as "No single diagnosis". The following diagnoses were made based on clinical history provided and conventional MRI scans alone (**Table 4**).

Table 4: Diagnoses using MRI alone

MRI Diagnosis	Frequency (%)
No single diagnosis	**48 (76.2)**
Low grade glioma	4 (6.3)
High grade glioma	3 (4.8)
Metastasis	1 (1.6)
Meningioma	1 (1.6)
Schwannoma	1 (1.6)
Cerebral infarct	1 (1.6)
Radiation necrosis	1 (1.6)
Pilocytic astrocytoma	1 (1.6)
Cavernoma	2 (3.2)

Figure 19: Diagnoses using MRI alone



It is clear that from all patients referred for brain MRS, a significant proportion (76.2%) (95%CI 63.8-86.0%) were unable to get a single definite diagnosis based on MRI alone (**Figure 19**).

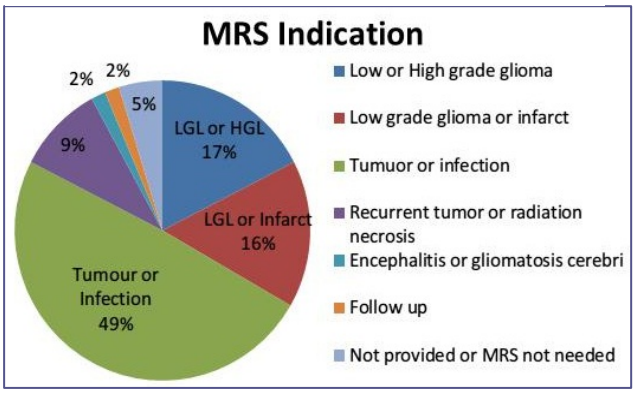
10.3. Indication for MRS

Table 5 lists the indications for which brain MR spectroscopies were performed during this study, which are represented in **Figure 20**.

Table 5: Indications for performing brain MR spectroscopy

MRS Indication	Frequency (%)
Low or High grade glioma	11 (17.5)
Low grade glioma or infarct	10 (15.9)
Tumour or infection	**31 (49.2)**
Recurrent tumour or radiation necrosis	6 (9.5)
Encephalitis or gliomatosis cerebri	1 (1.6)
Follow up	1 (1.6)
Not provided or not needed	3 (4.8)

Figure 20: MRS Indication



Tumour v/s Infection	31 of 63 (49.2%)
Tuberculoma v/s Glioblastoma Multiforme	30 of 31(96.7%)(p<0.001)
Toxoplasmosis v/s Primary CNS Lymphoma	1 of 31(3.3%)

The largest proportion of brain MR spectroscopies (49.2%) (p=0.899) were performed to help differentiate tumours from infections. Of these 31 indications, 30 (96.7%) (p<0.001) were performed to differentiate Tuberculoma, which is an infective lesion, from Glioblastoma Multiforme (GBM), which is a high grade glial tumour. The remaining indication from this sub-group was to differentiate Primary CNS Lymphoma from Toxoplasmosis in a patient with HIV infection. These statistics suggest that tuberculomas are statistically significantly the most common infectious intracranial mass lesions seen in this population. It is worth noting that no diagnosis of pyogenic abscess was seen either based on MRI alone nor MRI + MRS.

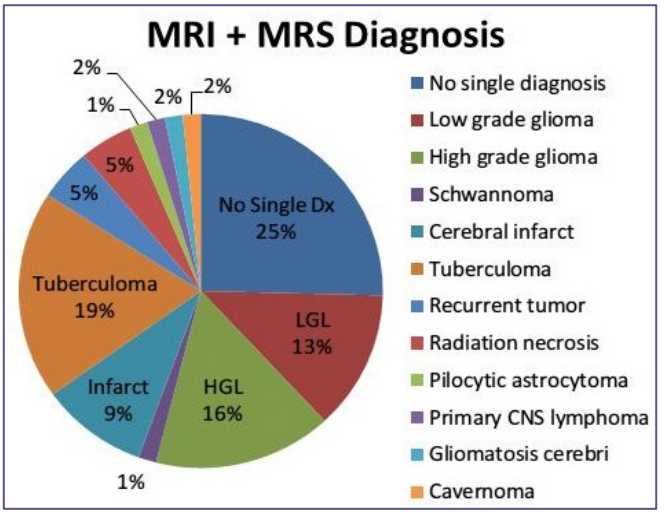
10.4. MRI + MRS Diagnosis

Table 6 provides the diagnoses and their frequencies that were made after correlating the MRI findings to the findings of single-voxel MR spectroscopy performed on the intracranial mass lesions. It is important to note that only definitive imaging diagnoses were recorded as such. If there was more than one differential, this was recorded as "no single diagnosis". These diagnoses were made by combining MRI and MRS findings, not from MRS alone. The two most frequent diagnoses made using MRI and MRS were High grade gliomas (15.9%) (95%CI 7.9-27.3%) and Tuberculomas (19%) (95%CI 10.3-30.9%).

Table 6: Diagnoses using MRI + MRS

MRI + MRS Diagnosis	Frequency (%)
No single diagnosis	**16 (25.4)**
Low grade glioma	8 (12.7)
High grade glioma	**10 (15.9)**
Schwannoma	1 (1.6)
Cerebral infarct	6 (9.5)
Tuberculoma	**12 (19.0)**
Recurrent tumour	3 (4.8)
Radiation necrosis	3 (4.8)
Pilocytic astrocytoma	1 (1.6)
Primary CNS lymphoma	1 (1.6)
Gliomatosis cerebri	1 (1.6)
Cavernoma	1 (1.6)

Figure 21: MRI + MRS Diagnosis



10.5. Efficacy of MRI + MRS

Table 7: Overall diagnostic performance between MRI Diagnosis and MRI+MRS Diagnosis

Findings	MRI Diagnosis n (%)	MRI + MRS Diagnosis n (%)	P value
Single Imaging Diagnosis	15 (23.8)	47 (74.6)	<0.001
No Single Imaging Diagnosis	48 (76.2)	16 (25.4)	

Of all patients examined by MRI and MRS for intracranial mass lesions, the radiologists were able to offer a single definite imaging diagnosis based on MRI alone in only 23.8% of patients while when MRI imaging was combined with MR spectroscopy, a single imaging diagnosis was offered in 74.6% of patients. This was an overall statistically significant improvement of 313.4% (P-value <0.001), a slightly more than 3-fold improvement in diagnostic performance (**Table 7**). It is important to note that the majority of this

population was biased towards patients who had been referred for MRS because of inconclusive prior imaging.

When we combine all individual diagnoses made using MRI alone and MRI + MRS, from all 63 patients, the addition of MRS improved the imaging diagnosis in 35 of these patients (55.6%). This means that the radiologist was able to rule out some differentials and offer a single imaging diagnosis, or was in a few cases, able to change the single diagnosis that was offered based on MRI alone. In 28 patients (44.4%), the addition of MRS was either not able to rule out other differentials or it did not change the single diagnosis that was offered based on MRI alone. Although this is an improvement, it is not statistically significant (p=0.374) (**Table 8**).

Table 8: Did MRS improve imaging diagnosis?

Did MRS Improve Imaging Diagnosis? n (%)		P-value
Yes	35 (55.6)	0.374
No	28 (44.4)	

Table 9: Comparison of MRI and MRI+MRS Diagnosis

Findings	MRI Diagnosis n (%)	MRI+MRS Diagnosis n (%)	P value
No diagnosis	48 (76.2)	16 (25.4)	<0.001
Low grade glioma	4 (6.3)	8 (12.7)	<0.001
High grade glioma	3 (4.8)	10 (15.9)	<0.001
Metastasis	1 (1.6)	0	
Meningioma	1 (1.6)	0	
Schwannoma	1 (1.6)	1 (1.6)	
Cerebral infarct	1 (1.6)	6 (9.5)	<0.001
Tuberculoma	0	12 (19.0)	<0.001
Recurrent tumour	0	3 (4.8)	<0.001
Radiation necrosis	1 (1.6)	3 (4.8)	<0.001
Pilocytic astrocytoma	1 (1.6)	1 (1.6)	
Primary CNS lymphoma	0	1 (1.6)	
Gliomatosis cerebri	0	1 (1.6)	
Cavernoma	2 (3.2)	1 (1.6)	

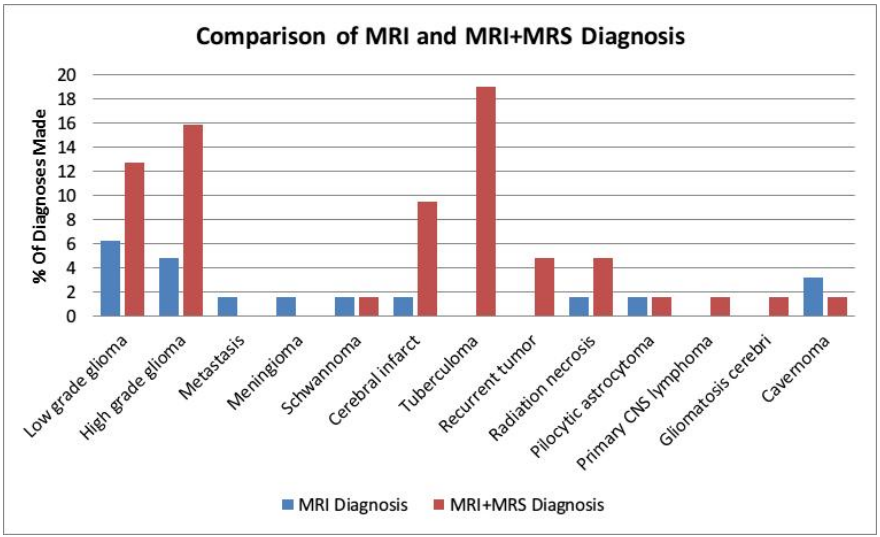


Figure 22: Comparison of MRI and MRI+MRS Diagnosis

The combination of MRI with MRS significantly increased the radiologist's chances of confidently diagnosing high grade gliomas, low grade gliomas, cerebral infarcts, tuberculomas, recurrent tumours and radiation necrosis, rather than with MRI alone (p<0.001 based on Wilcoxon Rank test). (**Table 9**), (**Figure 22**)

Table 10: MRS efficacy based on indication

Indication for MRS	Did MRS Improve Diagnosis ?		P value
	Yes	No	
Low or High grade glioma	6 (54.5%)	5 (45.5%)	1.000
Low grade glioma or Infarct	7 (70.0%)	3 (30.0%)	0.490
Tumour or Infection	16 (51.6%)	15 (48.4%)	0.535
Recurrent tumour or Radiation necrosis	5 (83.3%)	1 (16.7%)	0.214
Encephalitis or Gliomatosis cerebri	1 (100.0%)	0 (0.0%)	1.000

For the majority of indications for which MRS was utilised, addition of MRS to MRI improved the imaging diagnosis. However, the improvement was not statistically significant ($p>0.05$) (**Table 10**).

10.6. MRS Spectral Changes based on Diagnosis

Table 11 displays the metabolite spectral changes that were observed against the final imaging diagnosis, showing each metabolite and frequencies and percentages of those which were increased, decreased and those in which there was no change; for the major diagnoses. Further tables below compare the significance of differences between metabolite changes for the main MRS indications.

Table 11: Observed metabolite spectral changes. ↔ No change; ↑=Increased; ↓=Decreased.

Diagnosis	Change	NAA		Cr		Cho		Lip		Lac		Mi		Glx	
		n	%	n	%	n	%	n	%	n	%	N	%	n	%
Low grade glioma	↔	3	37.5	7	87.5	1	12.5	1	12.5	2	25	2	25	8	100
	↑	0	0	0	0	6	75	7	87.5	6	75	6	75	0	0
	↓	5	62.5	1	12.5	1	12.5	0	0	0	0	0	0	0	0
	P value		0.727		0.070		0.059		0.070		0.125		0.289		-
High grade glioma	↔	0	0	3	30	0	0	0	0	0	0	2	20	8	80
	↑	0	0	0	0	10	100	10	100	8	100	5	50	0	0
	↓	10	100	7	70	0	0	0	0	0	0	3	30	2	20
	P value		-		0.206		-		-		-		0.242		0.058
Cerebral infarct	↔	3	50	4	66.7	3	50	0	0	0	0	1	16.7	5	83.3
	↑	0	0	0	0	1	16.7	6	100	6	100	3	50	0	0
	↓	3	50	2	33.3	2	33.3	0	0	0	0	2	33.3	1	16.7
	P value		1		0.688		0.877		-		-		0.877		0.219
Tuberculoma	↔	0	0	6	50	0	0	0	0	5	41.7	6	50	10	83.3
	↑	0	0	0	0	10	83.3	12	100	7	58.3	0	0	0	0
	↓	12	100	6	50	2	16.7	0	0	0	0	6	50	2	16.7
	P value		-		1.000		0.021		-		-		1.000		0.021
Recurrent tumour	↔	0	0	2	66.7	0	0	1	33.3	1	33.3	0	0	3	100
	↑	0	0	0	0	3	100	2	66.7	2	66.7	3	100	0	0
	↓	3	100	1	33.3	0	0	0	0	0	0	0	0	0	0
	P value		-		1.000		-		1.000		1.000		-		-
Radiation necrosis	↔	0	0	1	33.3	2	66.7	0	0	0	0	0	0	2	66.7
	↑	0	0	0	0	0	0	3	100	3	100	2	66.7	0	0
	↓	3	100	2	66.7	1	33.3	0	0	0	0	1	33.3	1	33.3
	P value		-		1.000		1.000		-		-		1.000		1.000

Table 12: Observed metabolite changes in low grade v/s high grade gliomas

Metabolite	Change	Low grade glioma	High grade glioma	P value
NAA	↔	3 (37.5%)	0 (0.0%)	0.069
	↑	0 (0.0%)	0 (0.0%)	
	↓	5 (62.5%)	10 (100.0%)	
Cr	↔	7 (87.5%)	3 (30.0%)	0.025
	↑	0 (0.0%)	0 (0.0%)	
	↓	1 (12.5%)	7 (70.0%)	
Cho	↔	1 (12.5%)	0 (0.0%)	0.183
	↑	6 (75.0%)	10 (100.0%)	
	↓	1 (12.5%)	0 (0.0%)	
Lip	↔	1 (12.5%)	0 (0.0%)	0.444
	↑	7 (87.5%)	10 (100.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Lac	↔	1 (14.3%)	2 (20.0%)	0.467
	↑	6 (85.7%)	8 (80.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Mi	↔	2 (25.0%)	2 (20.0%)	0.614
	↑	6 (75.0%)	5 (50.0%)	
	↓	0 (0.0%)	3 (30.0%)	
Glx	↔	8 (100.0%)	8 (80.0%)	0.477
	↑	0 (0.0%)	0 (0.0%)	
	↓	0 (0.0%)	2 (20.0%)	

Table 13: Observed metabolite changes in low grade glioma v/s cerebral infarcts

Metabolite	Change	Low grade glioma	Cerebral infarct	P value
NAA	↔	3 (37.5%)	3 (50.0%)	1
	↑	0 (0.0%)	0 (0.0%)	
	↓	5 (62.5%)	3 (50.0%)	
Cr	↔	7 (87.5%)	4 (66.7%)	0.538
	↑	0 (0.0%)	0 (0.0%)	
	↓	1 (12.5%)	2 (33.3%)	
Cho	↔	1 (12.5%)	3 (50.0%)	0.149
	↑	6 (75.0%)	1 (16.7%)	
	↓	1 (12.5%)	2 (33.3%)	
Lip	↔	1 (12.5%)	0 (0.0%)	1
	↑	7 (87.5%)	6 (100.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Lac	↔	1 (14.3%)	0 (0.0%)	1
	↑	6 (85.7%)	6 (100.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Mi	↔	2 (25.0%)	1 (16.7%)	0.329
	↑	6 (75.0%)	3 (50.0%)	
	↓	0 (0.0%)	2 (33.3%)	
Glx	↔	8 (100.0%)	5 (83.3%)	0.429
	↑	0 (0.0%)	0 (0.0%)	
	↓	0 (0.0%)	1 (16.7%)	

Table 12 compares metabolite spectral changes between low grade and high grade gliomas. Of statistical significance was the fact that Creatine was depressed in 70% of lesions diagnosed as high grade glioma, while it was unchanged in all but one lesion diagnosed as low grade glioma (p=0.025).

Decreased NAA, increased Choline, Lipid and Lactate were seen in both grades of tumours, with no significant difference in observed spectral changes.

Table 13 compares metabolite spectral changes between low grade gliomas and cerebral infarcts. NAA was reduced and Creatine was unchanged in both. Choline was increased in 75% of low grade gliomas, while it was unchanged or decreased in 83.3% of cerebral infarcts. Although this trend is radiologically important for distinction between the two, the difference between the two groups was not statistically significant (p=0.149).

This is most likely due to the small frequency of these lesions, and a larger frequency would likely show a statistically significant difference. Lipid and Lactate were increased in both. Myo-inositol was increased in 75% of lesions diagnosed as low grade gliomas while in cerebral infarcts it was either increased, decreased or no change seen.

Table 14: Observed metabolite changes in recurrent tumour v/s radiation necrosis

Metabolite	Change	Recurrent tumor	Radiation necrosis	P value
NAA	↔	0 (0.0%)	0 (0.0%)	-
	↑	0 (0.0%)	0 (0.0%)	
	↓	3 (100.0%)	3 (100.0%)	
Cr	↔	2 (66.7%)	1 (33.3%)	1
	↑	0 (0.0%)	0 (0.0%)	
	↓	1 (33.3%)	2 (66.7%)	
Cho	↔	0 (0.0%)	2 (66.7%)	0.071
	↑	3 (100.0%)	0 (0.0%)	
	↓	0 (0.0%)	1 (33.3%)	
Lip	↔	1 (33.3%)	0 (0.0%)	1
	↑	2 (66.7%)	3 (100.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Lac	↔	1 (33.3%)	0 (0.0%)	1
	↑	2 (66.7%)	3 (100.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Mi	↔	3 (100.0%)	2 (66.7%)	1
	↑	0 (0.0%)	0 (0.0%)	
	↓	0 (0.0%)	1 (33.3%)	
Glx	↔	3 (00.0%)	2 (66.7%)	1
	↑	0 (0.0%)	0 (0.0%)	
	↓	0 (0.0%)	1 (33.3%)	

Table 15: Observed metabolite changes in high grade gliomas v/s tuberculomas

Metabolite	Change	High grade glioma	Tuberculoma	P value
NAA	↔	0 (0.0%)	0 (0.0%)	-
	↑	0 (0.0%)	0 (0.0%)	
	↓	10 (100.0%)	12 (100.0%)	
Cr	↔	3 (30.0%)	6 (50.0%)	0.415
	↑	0 (0.0%)	0 (0.0%)	
	↓	7 (70.0%)	6 (50.0%)	
Cho	↔	0 (0.0%)	0 (0.0%)	0.481
	↑	10 (100.0%)	10 (83.3%)	
	↓	0 (0.0%)	2 (16.7%)	
Lip	↔	0 (0.0%)	0 (0.0%)	-
	↑	10 (100.0%)	12 (100.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Lac	↔	0 (0.0%)	0 (0.0%)	-
	↑	8 (100.0%)	7 (100.0%)	
	↓	0 (0.0%)	0 (0.0%)	
Mi	↔	2 (20.0%)	6 (50.0%)	0.007
	↑	5 (50.0%)	0 (0.0%)	
	↓	3 (30.0%)	6 (50.0%)	
Glx	↔	8 (80.0%)	10 (83.3%)	1
	↑	0 (0.0%)	0 (0.0%)	
	↓	2 (20.0%)	2 (16.7%)	

Table 14 compares metabolite spectral changes between recurrent tumour and radiation necrosis. Due to the very small frequencies encountered of these lesions, no statistically significant differences in metabolite changes between the two groups was seen. However, from a radiological perspective, Choline was increased in all (100%) of recurrent tumours, while it was not increased in any diagnosis of radiation necrosis (p=0.071). Decreased NAA and increased Lipid and Lactate were seen in both groups.

Table 15 compares metabolite spectral changes between high grade gliomas and tuberculomas. The only statistically significant difference between the two groups was that Myo-inositol was increased in 50% of high grade glioma diagnoses, while in all tuberculomas Myo-inositol was either unchanged or decreased (p=0.007). Decreased NAA, increased Choline, Lipid and Lactate were seen in both sets of diagnoses.

10.7. MRS Ratios based on Diagnosis

Table 16: MRS metabolite ratios according to diagnosis at TE 31 msec (above) and TE 144 msec (below). (ar) = peak area, (ht) = peak height, =Abnormally increased values, =Abnormally decreased values, =Significant P values

Metabolite Ratio TE 31 msec		Low grade glioma	High grade glioma	Cerebral infarct	Tuberculoma	Recurrent tumour	Radiation necrosis	P value
Cho:NAA (ar)	Mean	0.78	1.80	3.30	1.37	1.93	3.43	0.514
	SD	0.34	1.07	6.32	0.90	1.48	5.08	
Cho:NAA (ht)	Mean	1.02	2.15	3.30	1.40	2.16	1.07	0.544
	SD	0.42	0.70	6.32	0.73	1.69	0.94	
NAA:Cho (ar)	Mean	1.47	0.68	1.71	1.70	0.46	1.40	0.652
	SD	0.54	0.29	0.93	2.76	0.62	1.16	
NAA:Cho (ht)	Mean	1.18	0.51	1.71	1.35	0.40	1.43	0.371
	SD	0.62	0.17	0.95	1.94	0.60	0.88	
Cho:Cr (ar)	Mean	1.20	3.18	2.86	2.79	3.41	25.37	0.031
	SD	0.39	1.31	3.85	1.68	2.74	41.27	
Cho:Cr (ht)	Mean	1.21	2.74	1.29	3.57	2.63	1.92	0.695
	SD	0.21	0.96	1.03	6.05	1.40	1. 52	
NAA:Cr (ar)	Mean	1.82	2.14	2.04	2.91	0.69	4.87	0.301
	SD	0.92	0.99	1.11	3.73	1.29	3.20	
NAA:Cr (ht)	Mean	1.39	1.40	1.47	2.06	0.53	1.96	0.584
	SD	0.61	0.57	0.83	2.25	1.02	0.85	

Metabolite Ratio TE 144 msec		Low grade glioma	High grade glioma	Cerebral infarct	Tuberculoma	Recurrent tumour	Radiation necrosis	P value
Cho:NAA (ar)	Mean	3.22	3.88	1.56	1.80	1.86	1.67	0.399
	SD	5.28	1.51	1.73	0.43	0.78	1.07	
Cho:NAA (ht)	Mean	1.48	3.88	1.19	1.80	1.81	1.02	<0.001
	SD	0.68	1.40	1.23	0.43	1.00	0.38	
Cho:Cr (ar)	Mean	1.62	3.19	4.81	2.35	1.71	2.64	0.621
	SD	0.46	1.18	8.86	0.43	1.02	2.62	
Cho:Cr (ht)	Mean	1.63	3.30	2.62	2.13	1.78	2.48	0.331
	SD	0.36	1.26	3.46	0.28	0.84	2.71	
NAA:Cho (ar)	Mean	0.84	0.29	1.26	0.46	0.36	0.81	0.009
	SD	0.60	0.10	0.76	0.47	0.45	0.53	
NAA:Cho (ht)	Mean	0.84	0.29	1.26	0.44	0.38	1.11	0.001
	SD	0.43	0.10	0.51	0.53	0.54	0.53	
NAA:Cr (ar)	Mean	1.39	0.88	3.16	1.38	0.81	1.32	0.116
	SD	0.52	0.25	3.88	0.38	0.79	0.61	
NAA:Cr (ht)	Mean	1.36	0.89	3.41	1.22	0.82	2.13	0.085
	SD	0.52	0.23	4.11	0.22	0.89	1.95	

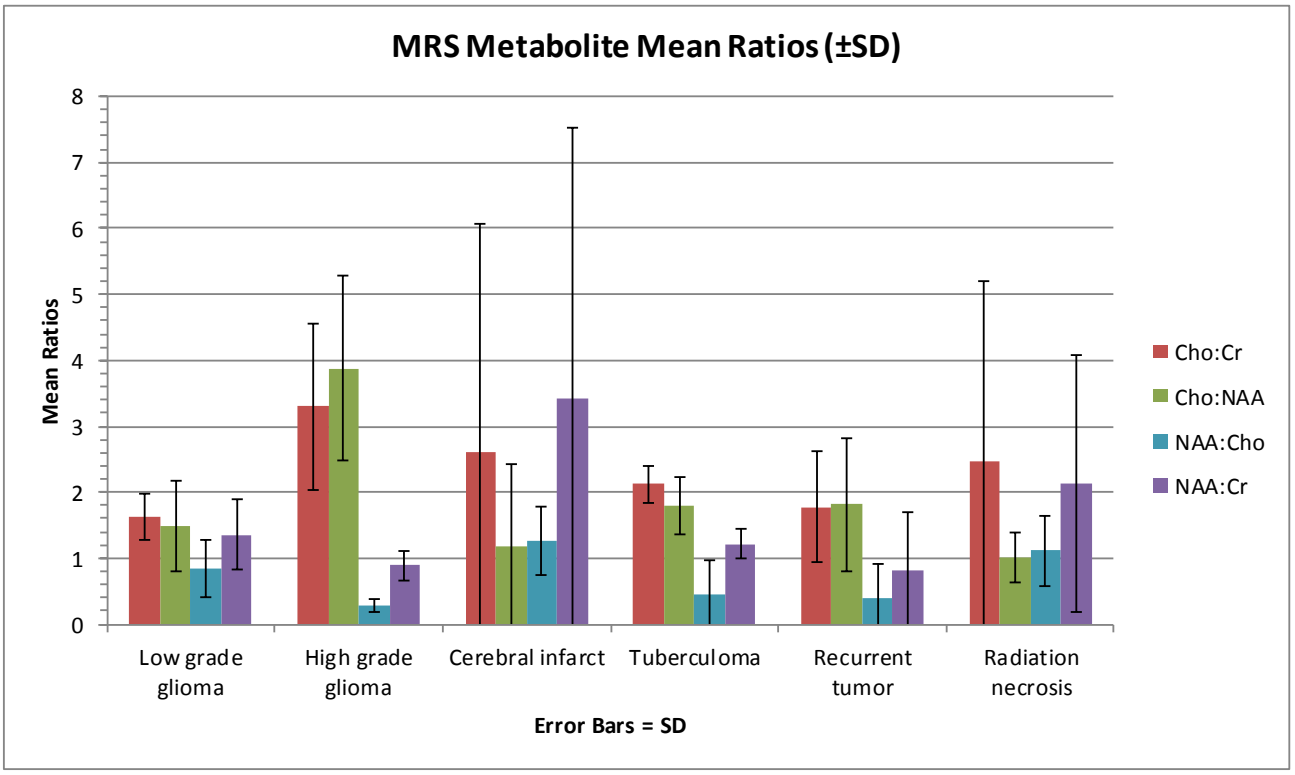


Figure 23: MRS metabolite mean ratios at TE 144 msec (± Standard deviation)

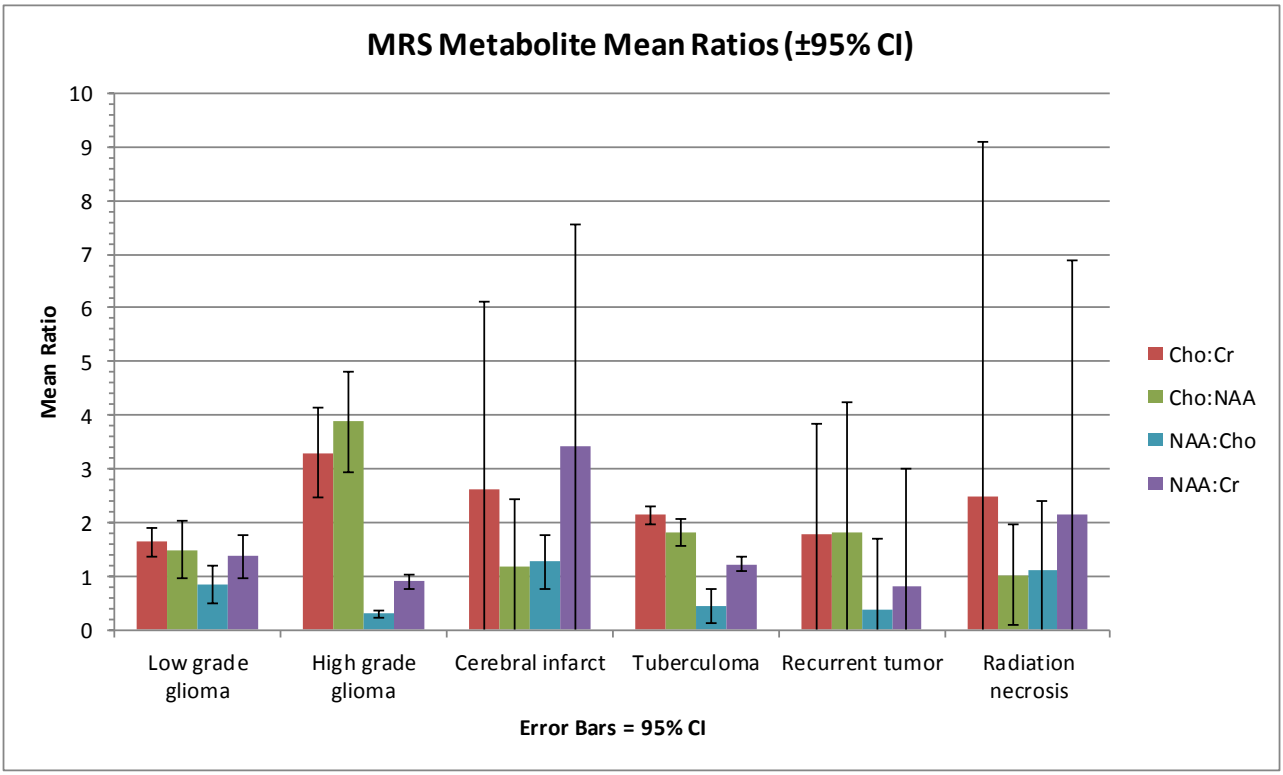


Figure 24: MRS metabolite mean ratios at TE 144 msec (±95% Confidence interval). Error bars which are not overlapping show significant differences between the groups.

Table 16, **Figure 23** and **Figure 24** provide the metabolite ratios tabulated against the major MRS diagnoses. For the statistical analysis, ratios of peak areas were disregarded due to exaggerated erroneous peak areas in a number of cases caused by curve smoothing by the built-in MRS software of the

equipment. As Creatine (Cr) was used as a reference, decreased and near zero Cr values caused erroneously elevated ratios in some cases e.g. Cho:Cr (ar) of Radiation Necrosis at TE 31. This was also excluded from the analysis. As Choline (Cho) is elaborated better at TE 144 msec, ratios were analysed based on TE 144 msec.

Cho:Cr and Cho:NAA (at both TE 31 and 144 msec) were significantly higher in patients with high grade glioma than in those with low grade glioma. Conversely, NAA:Cho and NAA:Cr were significantly lower in patients with high grade glioma than in those with low grade glioma.

Similarly, patients with high grade gliomas, tuberculomas and recurrent tumours had significantly lower NAA:Cho ratios. NAA:Cr was increased in cerebral infarcts and radiation necrosis due to Creatine depletion, while it was decreased in all other diagnoses. While NAA:Cr was decreased in both high grade gliomas and tuberculomas, the decrease in high grade gliomas was significantly more than in tuberculomas. Further analysis based on pairs of major diagnoses is provided below.

Table 17: Comparison of metabolite ratios between low grade and high grade gliomas.
■ =Abnormally increased values, ■ =Abnormally decreased values, ■ =Significant P values

MetaboliteRatio (TE)	Low grade glioma Mean (SD)	High grade glioma Mean (SD)	P value
NAA:Cr (31)	1.39 (0.61)	1.40 (0 .57)	0.975
NAA:Cho (31)	1.18 (0.62)	0.51 (0.17)	0.004
Cho:Cr (31)	1.21 (0.21)	2.74 (0.96)	<0.001
Cho:NAA (31)	1.02 (0.42)	2.15 (0.70)	0.001
NAA:Cr (144)	1.36 (0.52)	0.89 (0.23)	0.022
NAA:Cho (144)	0.84 (0.44)	0.29 (0.10)	0.001
Cho:Cr (144)	1.63 (0.36)	3.30 (1.26)	0.004
Cho:NAA (144)	1.48 (0.68)	3.88 (1.40)	<0.001

At TE 144 msec, all metabolite ratios showed statistically significant differences between low grade gliomas and high grade gliomas ($p<0.05$). NAA:Cr was reduced in both groups, however at TE 31 msec the difference was not statistically significant ($p>0.005$). Cho:Cr and Cho:NAA were much higher in high grade gliomas, while NAA:Cr and NAA:Cho were much lower (**Table 17**).

MRS was also useful in differentiating some tuberculomas from high grade gliomas. Although increased Choline was seen in both, Cho:Cr and Cho:NAA were significantly much higher in high grade gliomas than in those confidently diagnosed as tuberculomas. NAA:Cr and NAA:Cho were reduced in both, however, only the reduction in NAA:Cr was statistically significantly more in high grade gliomas than in tuberculomas (at TE 144 msec) (**Table 18**).

Table 18: Comparison of metabolite ratios between high grade gliomas and tuberculomas.
■ =Abnormally increased values, ■ =Abnormally decreased values, ■ =Significant P values

Metabolite Ratio (TE)	High grade glioma Mean (SD)	Tuberculoma Mean (SD)	P value
NAA:Cr (31)	1.40 (0.57)	2.06 (2.25)	0.374
NAA:Cho (31)	0.51 (0.17)	1.35 (1.94)	0.189
Cho:Cr (31)	2.74 (0.96)	3.57 (6.05)	0.671
Cho:NAA (31)	2.15 (0.70)	1.40 (0.73)	0.022
NAA:Cr (144)	0.89 (0.23)	1.22 (0.22)	0.003
NAA:Cho (144)	0.29 (0.10)	0.44 (0.53)	0.391
Cho:Cr (144)	3.30 (1.26)	2.13 (0.28)	0.007
Cho:NAA (144)	3.88 (1.40)	1.80 (0.43)	<0.001

Brain MRS was also used to help differentiate low grade gliomas from cerebral infarcts. The low frequency of cerebral infarcts diagnosed and the wide variation in ratios encountered meant that no statistically significant differences were seen between the two groups. However, NAA:Cr and NAA:Cho were much lower in low grade gliomas compared to infarcts. The markedly reduced (almost zero) values of NAA and Creatine seen in several diagnosed cerebral infarcts (wide SD), caused deceptively elevated Cho:NAA (TE 31) and NAA:Cr and Cho:Cr ratios (TE 144)(**Table 19**).

Table 19: Comparison of metabolite ratios between low grade gliomas and cerebral infarcts.
■ =Abnormally increased values, ■ =Abnormally decreased values, ■ =Significant P values

Metabolite Ratio (TE)	Low grade glioma Mean (SD)	Cerebral infarct Mean (SD)	P value
NAA:Cr (31)	1.39 (0.61)	1.47 (0.83)	0.833
NAA:Cho (31)	1.18 (0.62)	1.71 (0.95)	0.240
Cho:Cr (31)	1.21 (0.21)	1.29 (1.03)	0.826
Cho:NAA (31)	1.02 (0.42)	3.30 (6.32)	0.317
NAA:Cr (144)	1.36 (0.52)	3.41 (4.11)	0.211
NAA:Cho (144)	0.84 (0.44)	1.26 (0.52)	0.119
Cho:Cr (144)	1.63 (0.36)	2.62 (3.46)	0.463
Cho:NAA (144)	1.48 (0.68)	1.19 (1.23)	0.586

A number of diagnoses of recurrent tumour v/s radiation necrosis were made based on MRS findings. No statistically significant differences between the two groups were seen due to low frequency of the occurrences. However, of note is the trend that NAA:Cr and NAA:Cho were much lower in recurrent tumour than in radiation necrosis; and Cho:NAA was much higher in recurrent tumour than in radiation necrosis. The very low levels of Creatine in radiation necrosis caused deceptively elevated Cho:Cr ratios (**Table 20**).

Table 20: Comparison of metabolite ratios between recurrent tumour and radiation necrosis.
■ =Abnormally increased values, ■ =Abnormally decreased values, ■ =Significant P values

Metabolite Ratio (TE)	Recurrent tumour Mean (SD)	Radiation necrosis Mean (SD)	P value
NAA:Cr (31)	0.53 (1.02)	1.96 (0.85)	0.135
NAA:Cho (31)	0.40 (0.60)	1.43 (0.88)	0.170
Cho:Cr (31)	2.63 (1.40)	1.92 (1.52)	0.583
Cho:NAA (31)	2.16 (1.69)	1.07 (0.94)	0.409
NAA:Cr (144)	0.82 (0.89)	2.13 (1.95)	0.348
NAA:Cho (144)	0.38 (0.54)	1.11 (0.53)	0.172
Cho:Cr (144)	1.78 (0.84)	2.48 (2.71)	0.693
Cho:NAA (144)	1.81 (1.00)	1.02 (0.39)	0.282

10.8. Selected MRI/MRS Images from Study Sample

Images from selected cases representing the spectrum of findings encountered during the study are presented below. All images shown are from actual cases of patients recruited in the study.

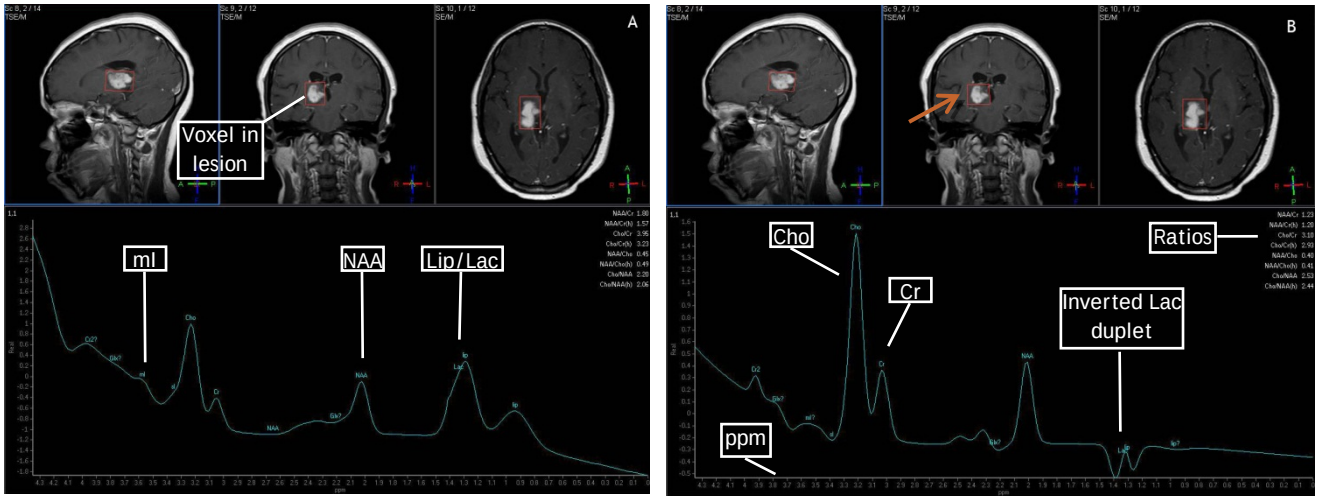


Figure 25: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between high grade and low grade glioma. Lesion was diagnosed as a high grade glioma. NAA is decreased. Lipid and Lactate are increased. Choline is markedly elevated. Cho:Cr ratio is 2.93, Cho:NAA ratio is 2.44. At TE31 Myo-inositol is reduced. Myo-inositol decrease has been associated with high grade gliomas (58).

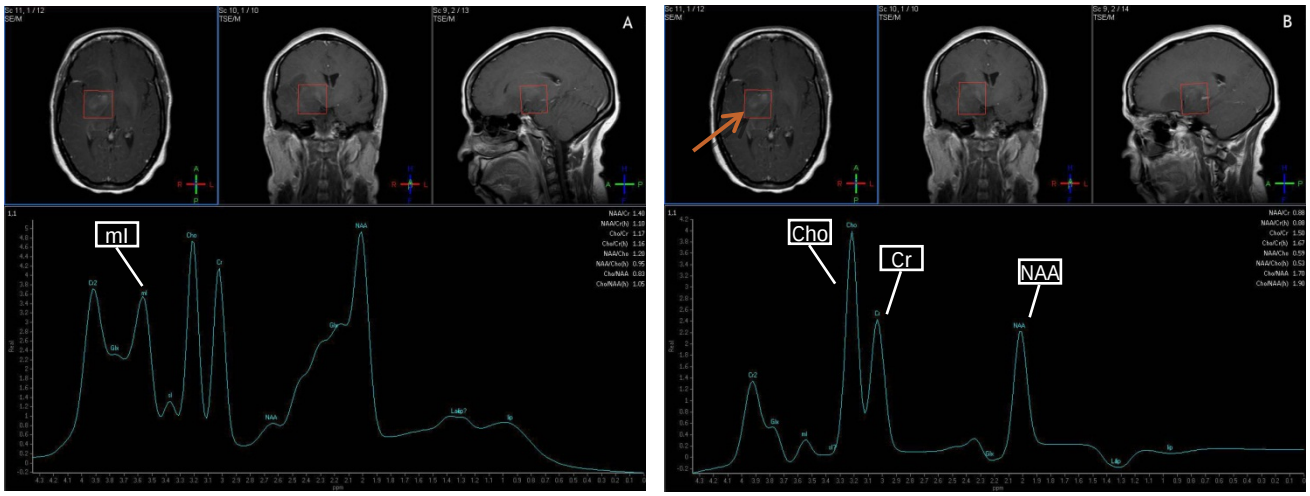


Figure 26: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between high grade and low grade glioma. Lesion was diagnosed as a low grade glioma. NAA is depressed. Choline is moderately elevated. Cho:Cr is 1.67, Cho:NAA is 1.90. Increased Myo-inositol is also seen at TE31.

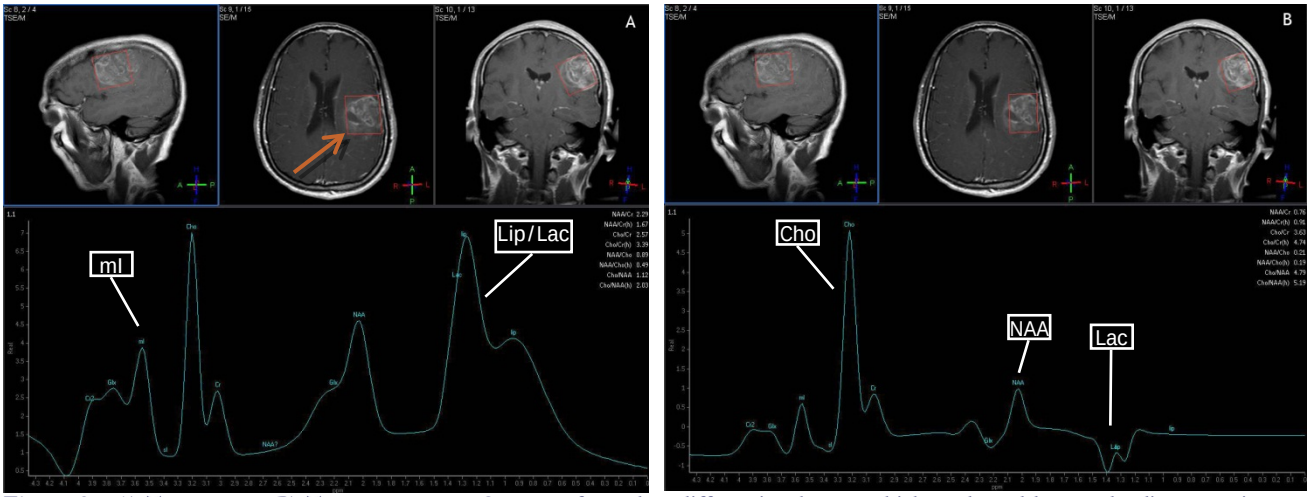


Figure 27: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between high grade and low grade glioma. Lesion was diagnosed as a high grade glioma. NAA is reduced. Lipid and Lactate are elevated. Choline is markedly elevated. Cho:Cr is 4.74, Cho:NAA is 5.19. However, at TE31, Myo-inositol is slightly increased. Myo-inositol increase has been associated with low grade gliomas (58).

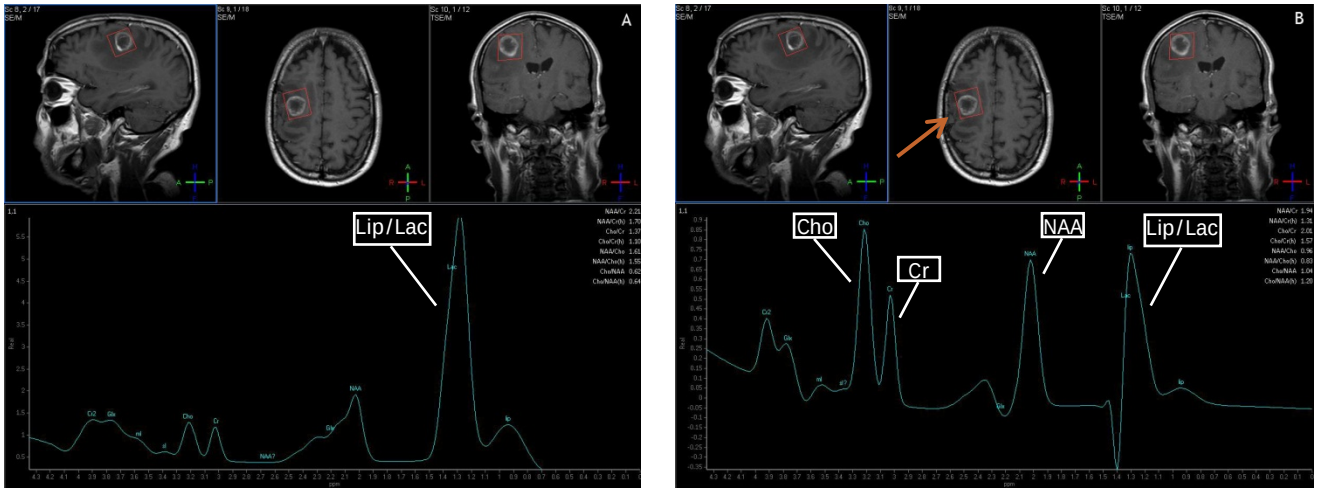


Figure 28: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between high grade glioma and tuberculoma. Lesion was diagnosed as a tuberculoma. At TE31 prominent Lipid peak is seen at 1.3ppm. At TE144 Choline is increased. However, Cho:Cr is 1.57 and Cho:NAA is 1.20.

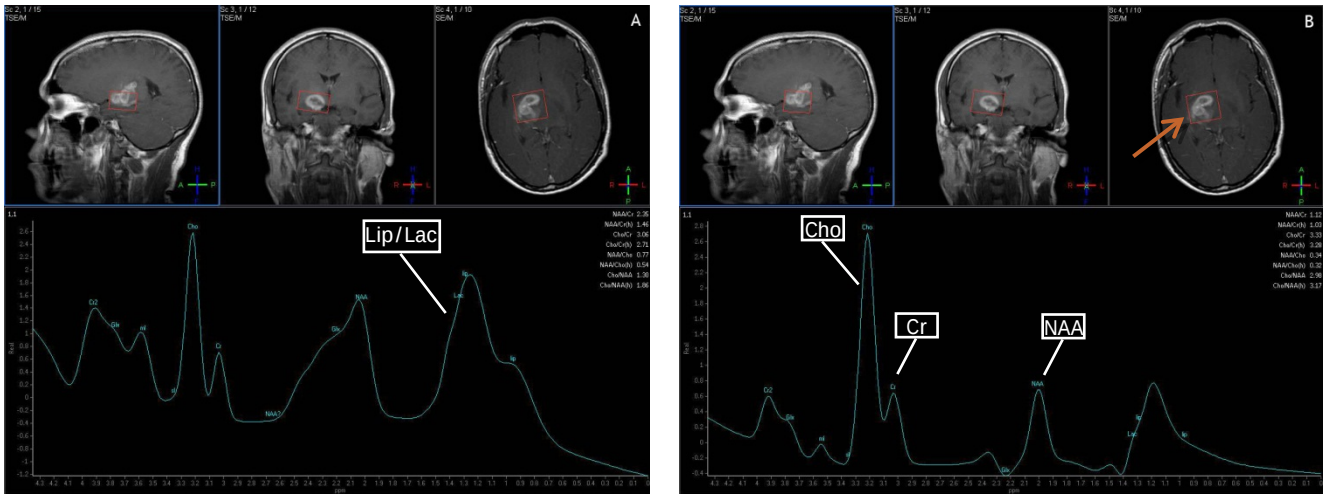


Figure 29: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between high grade glioma and tuberculoma. Lesion was diagnosed as a high grade glioma. NAA is depressed. Lipid is elevated. Choline is markedly elevated. Cho:Cr is 3.28, Cho:NAA is 3.17. Note that in this case diagnosed as high grade glioma, Myo-inositol at TE31 is not decreased.

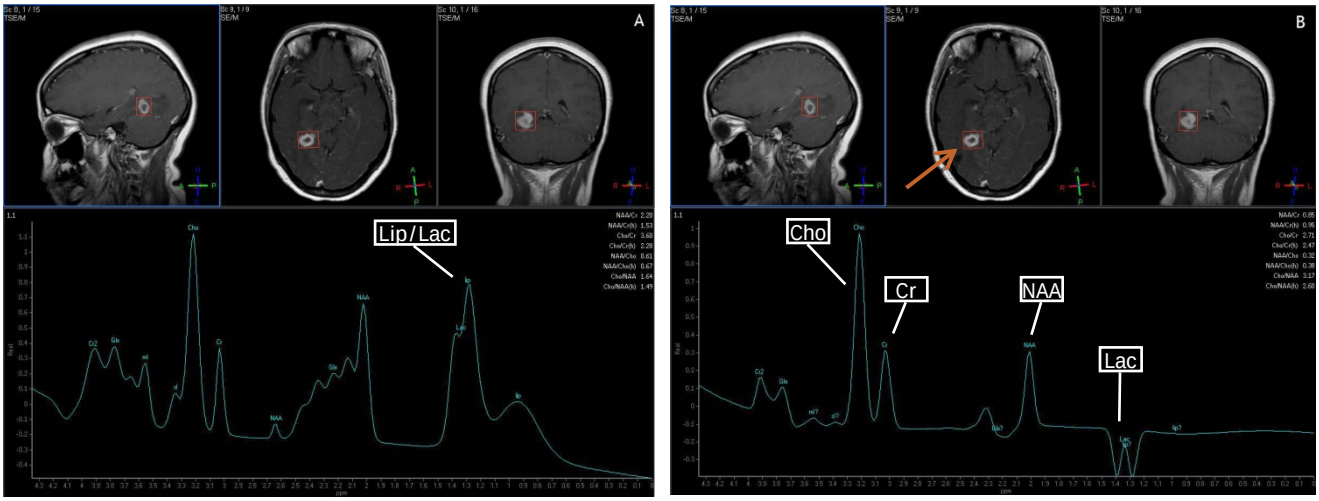


Figure 30: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between high grade glioma and tuberculoma. 'No single diagnosis' was offered. Lipids were elevated. Choline was elevated. Cho:Cr was 2.47, Cho:NAA was 2.60. Although this favours a diagnosis of high grade glioma, tuberculoma cannot be ruled out.

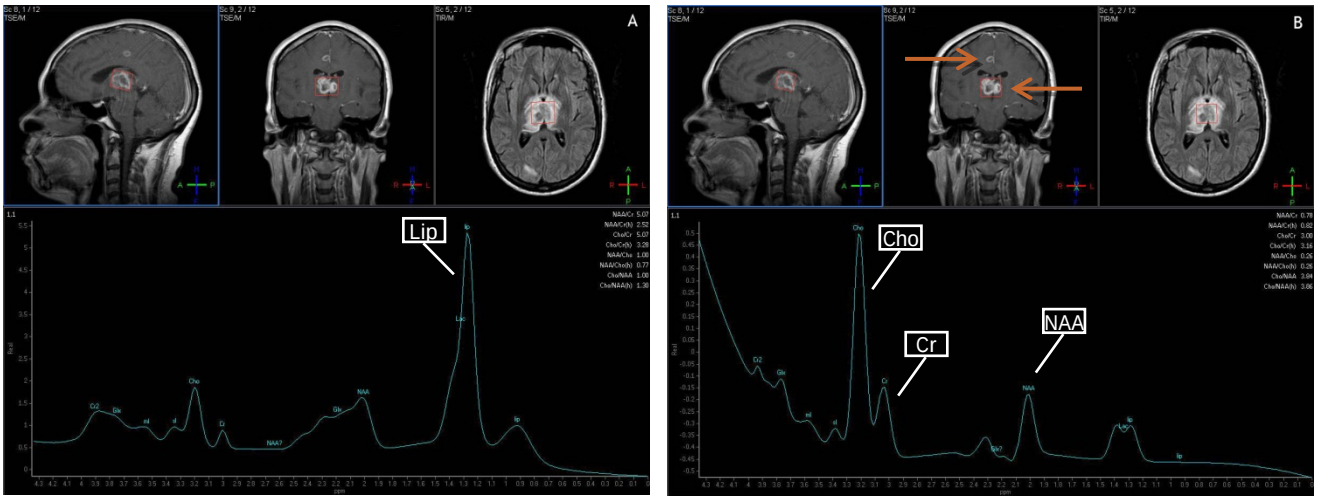


Figure 31: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between high grade glioma and tuberculoma. 'No single diagnosis' was offered. Multiple lesions with prominent Lipid peak at TE31 with other metabolites depressed favours a diagnosis of tuberculoma. But the markedly elevated Choline at TE144, Cho:Cr of 3.16 and Cho:NAA of 3.86 suggests an aggressive tumour. Metastasis cannot be ruled out.

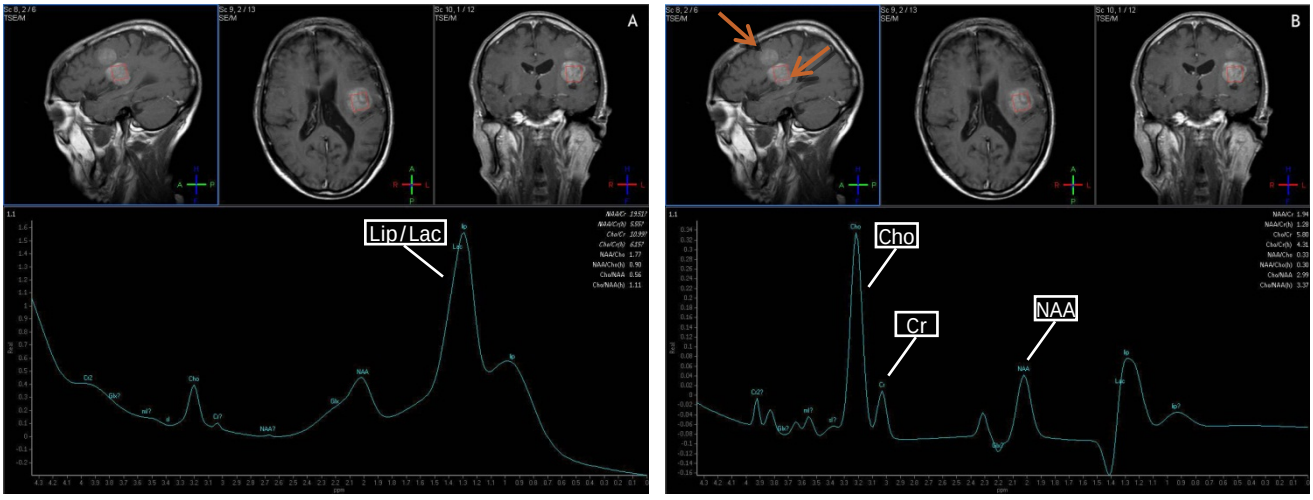


Figure 32: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between primary CNS lymphoma and toxoplasmosis. Lesion was diagnosed as multifocal primary CNS lymphoma. There is a prominent Lipid peak at TE31 which is also seen in toxoplasmosis. However, Choline is markedly elevated, Cho:Cr is 4.31 and Cho:NAA is 3.37. This suggests an aggressive tumour, hence the diagnosis.

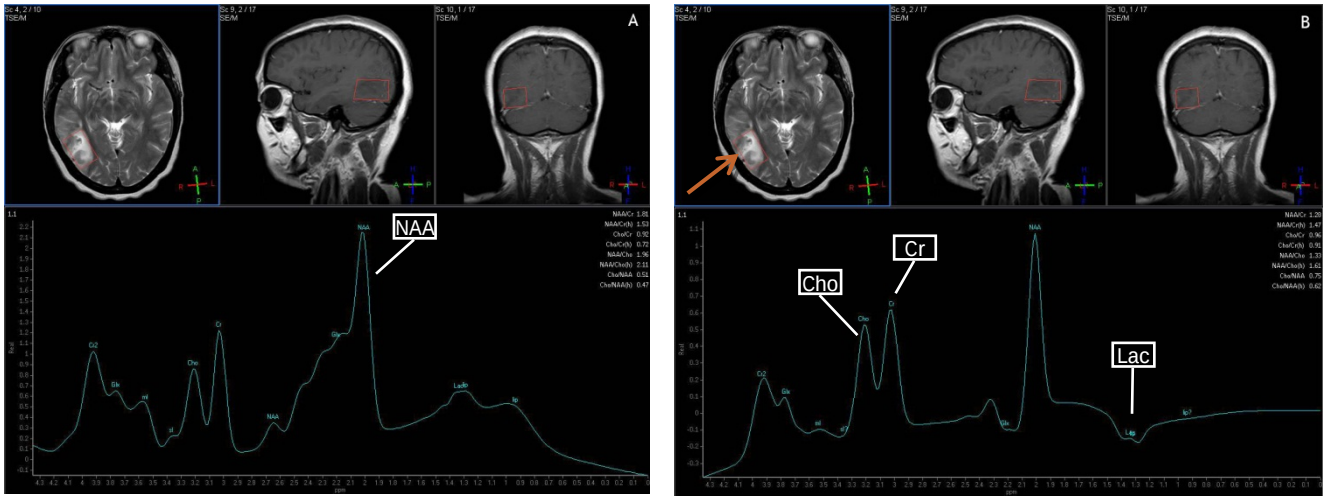


Figure 33: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between low grade glioma and cerebral infarct. Lesion was diagnosed as cerebral infarct. NAA is mildly reduced. Lactate is mildly increased. Choline and Creatine are normal. Cho:Cr (0.91) and Cho:NAA (0.62) were well within normal.

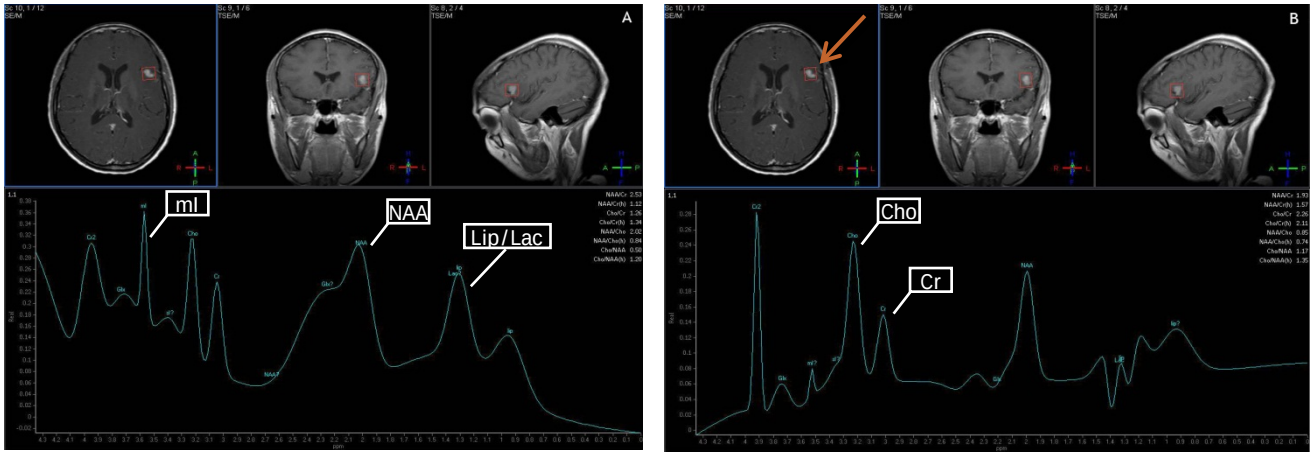


Figure 34: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between low grade glioma and cerebral infarct. Lesion was diagnosed as low grade glioma. NAA is depressed. Choline, Lipid, Lactate and Myo-inositol are elevated. Cho:Cr (2.11) and Cho:NAA (1.35) are mildly increased. NAA:Cho (0.74) is reduced.

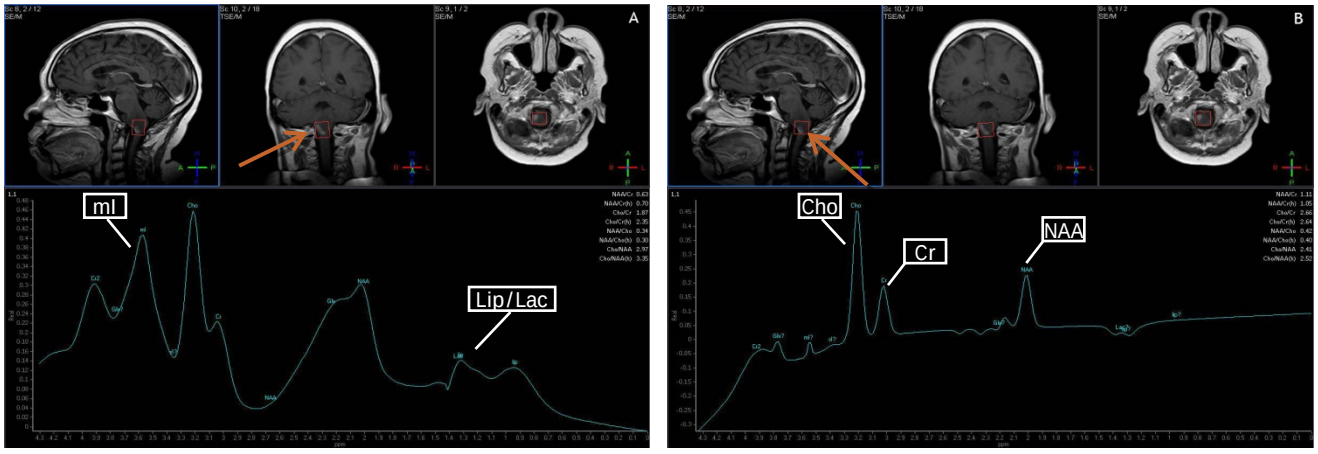


Figure 35: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between recurrent tumour and radiation necrosis. Lesion was diagnosed as recurrent tumour. MRS voxel placed over region of suspicious enhancement. NAA is depressed. Choline is elevated. Cho:Cr (2.64) and Cho:NAA (2.52) are increased. NAA:Cho (0.40) is reduced. Myo-inositol and mild Lipid/Lactate increase is also seen.

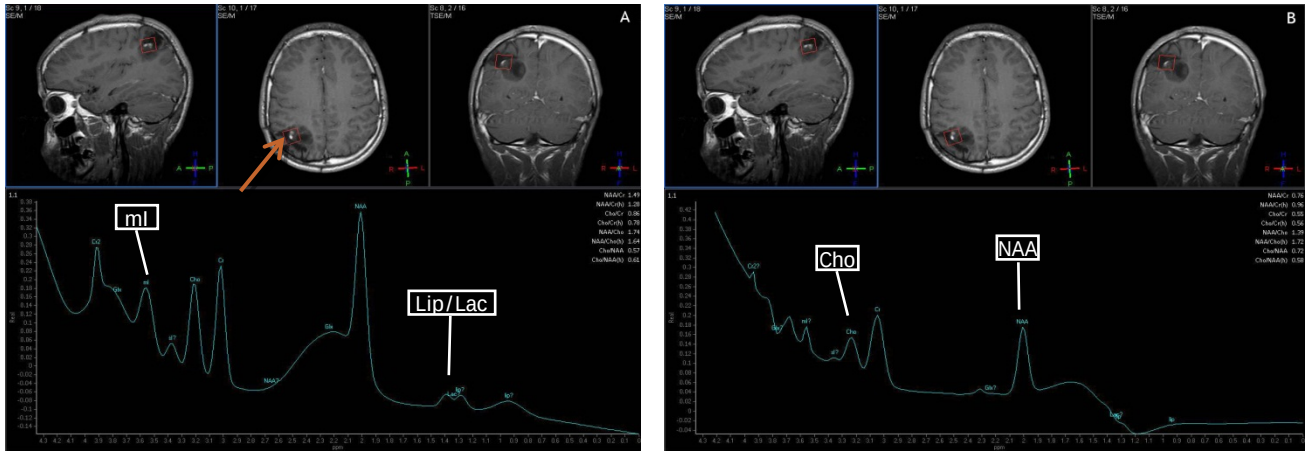


Figure 36: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between recurrent tumour and radiation necrosis. Lesion was diagnosed as radiation necrosis. MRS voxel placed over region of suspicious enhancement. NAA is mildly depressed. Lipid, Lactate and Myo-inositol are mildly elevated. Choline is not elevated. Cho:Cr (0.56), Cho:NAA (0.58) and NAA:Cho (1.72) are within normal.

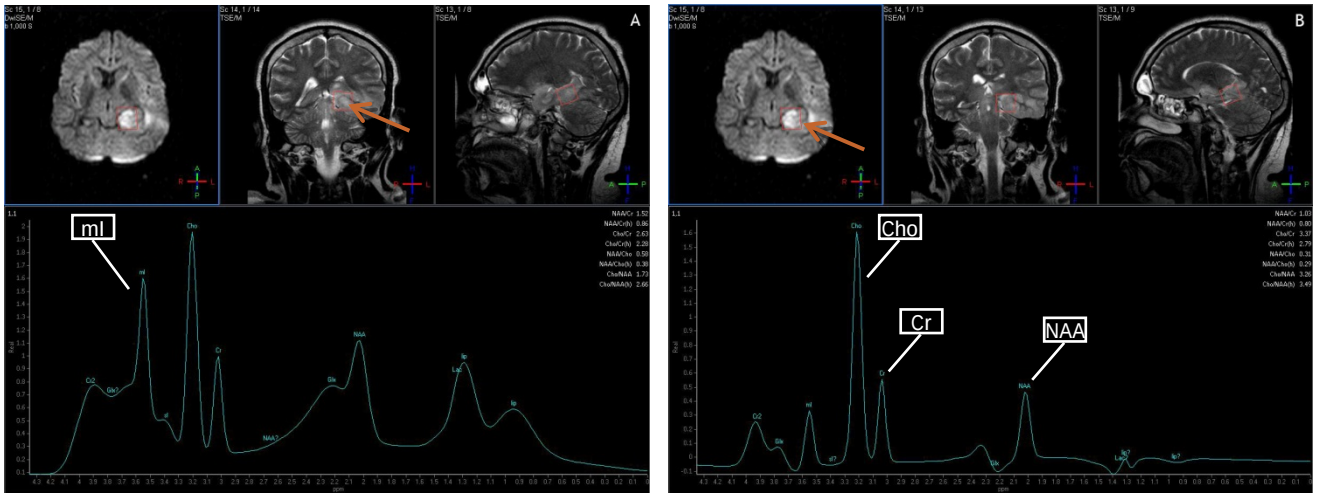


Figure 37: A) TE 31 msec. B) TE 144 msec. MRS was performed to differentiate between encephalitis and gliomatosis cerebri. Lesion was diagnosed as gliomatosis cerebri showing classical features of increased Myo-inositol, together with markedly elevated Choline.

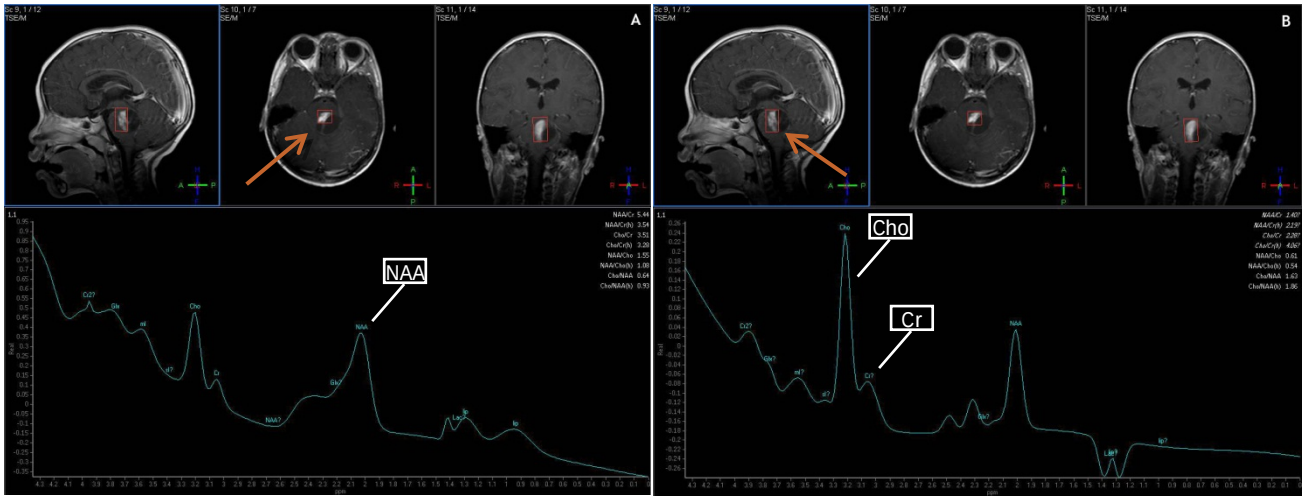


Figure 38: A) TE 31 msec. B) TE 144 msec. MRI diagnosis was pilocytic astrocytoma. MRS only confirmed accepted paradoxical findings of an aggressive-appearing metabolite pattern; increased choline and lactate; which do not reflect the histologically benign nature of this tumour.

11. Discussion

Overall, the impact of combining MRI with MRS in improving the radiologist's ability to offer a single imaging diagnosis was significant. The number of 'single diagnosis' increased 3-fold (in 47/63 patients (74.6%) with MRI+MRS compared to MRI alone (15/63 patients (23.8%). The most notable lesions, for which MRS aided the radiologist in offering a single diagnosis, were: high and low grade gliomas, tuberculomas, cerebral infarcts, recurrent tumours and radiation necrosis. It is important to note that the majority of this sample population was biased towards patients who had been referred for MRS because of inconclusive prior imaging. This shows that among the referring clinicians there is awareness about the utility of and indications for brain MRS in diagnosis of intracranial mass lesions.

However, if we consider whether or not brain MRS actually improved the imaging diagnosis, the results are not statistically significant. From all 63 patients, the addition of MRS improved the imaging diagnosis in just over half of these patients (55.6%). Although this is an improvement, it is not statistically significant ($p=0.374$). 'Improvement' was based on whether or not a single imaging diagnosis was obtainable after MRS in a patient where using MRI alone it was not.

Most patients presenting for brain MRS for intracranial mass lesions were in the age group 41-50 years. By far the largest number of brain MR spectroscopy examinations were performed to differentiate tuberculomas from high grade gliomas, seeing that both may present as ring-enhancing mass lesions. We can infer that the age group of 41-50 years is like a watershed group which presents more of a challenge when differentiating tuberculomas from high grade gliomas, because epidemiologically high grade gliomas tend to peak more in elderly patients, while tuberculomas tend to peak in younger patients(38,57).

Many times the request for brain MRS provided by the clinician indicated the reason or indication for performing MRS, either from findings of previous imaging or based on clinical findings. At times the indication for performing brain MRS was determined based on the differential diagnoses provided by the radiologist based on MRI findings. A few times, the clinician had requested for MRS examination, and the indication was not provided or determined. At times the radiologist did not see the need for an MRS examination based on a definite MRI diagnosis, but MRS was performed nonetheless as requested to confirm the MRI diagnosis.

Four major indications for brain MRS emerged from this study. These were: to differentiate low and high grade gliomas; to differentiate tumours from infections (96.7% of these were to differentiate high grade gliomas from tuberculomas); to differentiate low grade gliomas from cerebral infarcts; and to differentiate recurrent tumour from radiation necrosis.

If we look at the efficacy of MRS added to MRI in improving the imaging diagnosis v/s the four major indications described above, addition of MRS to MRI did indeed improve the imaging diagnosis in all four indications. However, the improvement was not statistically significant ($p>0.05$). It is possible that a larger sample size would probably show more statistically significant improvements.

When trying to differentiate between low grade and high grade gliomas, decreased NAA, increased Choline, Lipid and Lactate were seen in both grades of tumours, with no significant difference in observed spectral changes. Of statistical significance was the fact that Creatine was depressed in a large proportion of diagnosed high grade gliomas, while it remained unchanged in low grade glioma diagnoses ($p=0.025$).

Of important note is that Myo-Inositol was increased in 87.5% of lesions diagnosed as low grade gliomas. However, in lesions diagnosed as high grade glioma, Myo-Inositol was decreased in only 30% of lesions,

remained unchanged in 20%, and was increased in 50%. This contrasts the findings of Castillo et al, who reported a trend towards lower Myo-Inositol levels in high grade gliomas compared to increased levels in those of low-grade gliomas(58).

More significant differences between low and high grade glioma diagnoses were seen when we look at metabolite ratios. Cho:Cr and Cho:NAA ratios were significantly higher in patients with high grade glioma than in those with low grade glioma. Conversely, NAA:Cho and NAA:Cr were significantly lower in patients with high grade glioma than in those with low grade glioma. Mean Cho:Cr and Cho:NAA ratios were <2.00 in low grade gliomas – [1.63 (±0.36)] and [1.48 (±0.68)] respectively, while they were >2.00 in high grade gliomas – [3.30 (±1.26)] and [3.88 (±1.40)] respectively. Mean NAA:Cr was <1.80 in low grade gliomas [1.36 (±0.52)] and <1.00 in high grade gliomas [0.89 (±0.23)], while mean NAA:Cho was <1.20 in low grade gliomas [0.84 (±0.44)], and <0.50 in high grade gliomas [±0.29 (0.10)]. Our findings compare favourably with those of Bertholdo and Castillo et al who report that at different institutions the threshold for Cho:Cr and Cho:NAA for differentiating low and high grade gliomas ranges between 2.0 and 2.5(59).

The bulk of MRS examinations were performed to differentiate tuberculomas from high grade gliomas. Based on MR spectrum appearance, decreased NAA, increased Choline, Lipid and Lactate were seen in both sets of diagnoses. Early work by Gupta et al showed that tuberculomas tend to have prominent lipid peaks within the lesion, while all other metabolites are depressed(60). However, our findings in tuberculomas compare to more recent work by Gupta et al and Gutch et al which show that Choline increase is also seen in tuberculomas depending on the degree of cellular infiltrates and caseation(61,62).

The only statistically significant difference between the two groups was that Myo-inositol was increased in 50% of high grade glioma diagnoses, while in all tuberculomas Myo-inositol was either unchanged or decreased ($p=0.007$).

Differentiating tuberculomas from high grade gliomas can be particularly challenging based on the observed spectral changes of single-voxel MRS alone. Comparing metabolite ratios did assist in the differentiation. Although increased Choline was seen in both, Cho:Cr and Cho:NAA were significantly much higher in high grade gliomas,[3.30 (±1.26)] and [3.88 (±1.40)] respectively; than in those confidently diagnosed as tuberculomas [2.13 (±0.28)] and [1.80 (±0.43)]. NAA:Cr and NAA:Cho were reduced in both, however, only the reduction in NAA:Cr was statistically significantly more in high grade gliomas [0.89 (±0.23)] than in tuberculomas[1.22 (±0.22)].

31(49.2%) of MR spectroscopies were performed to differentiate tumours from non-tumours (infections). Of these, 30 (96.7%) were to differentiate tuberculomas from high grade gliomas. The lowly diagnostic performance of MRS in this regard(51.6% improvement of imaging diagnosis), occurred mainly when elevated Choline and Lipids were seen in a lesion, and the Cho:NAA and Cho:Cr ratios were such that they could not be confidently diagnosed as one or the other. A large number (48.4%) of such MR spectroscopies led to "No single diagnosis", with the both differentials still being considered. The age of the patients was also a factor in this diagnostic dilemma as explained above. It is in these situations where multi-voxel MRS if available, would have been particularly useful. Multi-voxel MRS would allow different parts of the lesion; the central area of breakdown, the enhancing solid rim, and the peri-tumoural region to be interrogated separately. Increased Choline in the peri-tumoural oedema would greatly increase the confidence of diagnosing a high grade glioma.

When multiple ring-enhancing lesions were encountered, this would favour tuberculomas; however, when such lesions had markedly elevated Choline and Cho:NAA and Cho:Cr ratios, it would be difficult to confidently diagnose tuberculomas, as a multifocal aggressive tumour like metastasis would also need to be considered in the differential.

Only one MRS was performed to differentiate between primary CNS lymphoma and toxoplasmosis in an HIV patient. No pyogenic abscess diagnosis was encountered.

The third indication for which MRS's were performed was to differentiate low grade gliomas from cerebral infarcts, in those whose prior imaging was inconclusive. Choline was increased in 75% of low grade gliomas, while it was unchanged or decreased in 83.3% of cerebral infarcts. Although this trend is radiologically important for distinction between the two, the difference between the two groups was not statistically significant ($p=0.149$). This is most likely due to the small frequency of these lesions encountered, and a larger frequency from a larger sample size would likely show a statistically significant difference. Another observation was that Myo-inositol was increased in low grade gliomas (75% of them), while in cerebral infarcts, no particular trend was seen; although without any statistical significance.

MRS metabolite ratios showed no statistically significant differences between the two groups. However, mean NAA:Cr and NAA:Cho ratios were much lower in low grade gliomas compared to infarcts. The markedly reduced (almost zero) values of NAA and Creatine seen in several diagnosed cerebral infarcts, caused deceptively elevated Cho:NAA (TE 31) and NAA:Cr and Cho:Cr ratios (TE 144). This suggests that the diagnosis of cerebral infarct v/s low grade glioma was more likely based on observed spectral changes of increased Choline in gliomas, rather than on metabolite ratios.

The indication for the fourth group of MRS examinations was to differentiate between recurrent tumour and radiation necrosis. Once again, due to the small frequencies encountered, no statistically significant differences in metabolite changes between the two groups were seen. However, from a radiological perspective, Choline was increased in all (100%) of recurrent tumours, while it was not increased in any diagnosis of radiation necrosis ($p=0.071$).

However, of note is the trend that NAA:Cr and NAA:Cho were much lower in recurrent tumour than in radiation necrosis; and Cho:NAA was much higher in recurrent tumour than in radiation necrosis. The very low levels of Creatine in radiation necrosis caused deceptively elevated Cho:Cr ratios.

One MRS examination was performed to help differentiate between encephalitis and gliomatosis cerebri which has a varied MRI appearance. MRS was useful in this instance as it showed typical MRS features of gliomatosis cerebri, which include elevated Myo-inositol together with features of aggressive tumour i.e. increased Choline, increased Cho:Cr and Cho:NAA ratios, and decreased NAA:Cr and NAA:Cho ratios(63).

One MRS examination was performed for a child whose MRI imaging diagnosis was a pilocytic astrocytoma. MRS findings only confirmed the MRI findings by showing typical accepted paradoxical findings of an aggressive-appearing metabolite pattern; increased choline and lactate; which do not reflect the histologically benign nature of this tumour(63). MRS in this case did not however improve the imaging diagnosis.

12. Limitations

One of the limitations of this study was the fact that we were unable to compare the imaging diagnosis with histopathological diagnosis; hence the investigator has referred to his findings as "imaging diagnosis" and not definitive diagnosis. As a result we were unable to provide local data on the sensitivity and specificity of MRS for diagnosis of intracranial mass lesions.

Secondly, while MRS findings showed trends in metabolite spectral changes and ratios for particular diagnoses and indication groups in one way or the other, the fact that the frequencies of several diagnoses encountered were small, it was difficult to determine the statistical significance of these trends. A larger sample size with longer study duration, or a study designed to target particular diagnoses would be required to resolve this.

An important factor to consider is that majority of patients recruited in this study may already have had prior imaging with inconclusive diagnosis. Due to cost implications, the decision to refer a patient for MRS is often made by the clinicians, either on their own, or upon recommendation of the radiologist. This, coupled with the fact that a consecutive series sample selection method was used; may have introduced an element of selection bias.

13. Conclusions

MRS combined with MRI is an important tool in the arsenal of a radiologist for diagnosing intracranial mass lesions, and provides a non-invasive way of interrogating the biochemical make-up of lesions within the brain. Overall, when patients are selectively chosen who have more than one differential based on conventional MRI, in whom MRS has the potential to answer the diagnostic question, MRS combined with MRI showed a three-fold increase in the number of single imaging diagnosis offered by the radiologist.

The most notable lesions, for which MRS aided the radiologist in offering a single diagnosis, were: high and low grade gliomas, tuberculomas, cerebral infarcts, recurrent tumours and radiation necrosis.

MRS combined with MRI 'improved' the imaging diagnosis in more than half of all patients examined. MRS also improved the imaging diagnosis in more than half of the patients within all four major indication groups. The improvement was however not statistically significant. We can conclude that MRS is useful in narrowing the MRI differential diagnoses to one diagnosis for several indications; however, this study could not demonstrate whether this improvement is significant; likely due to limitations of sample size.

MRS showed statistically significant value in differentiating low grade from high grade gliomas, based on depressed Creatine, and degree of increase in Cho:Cr and Cho:NAA ratios. The trend of Myo-inositol changes in both groups did not compare with those of other authors(58).

The largest number of MRS examinations was performed to differentiate tumours from non-tumorous lesions, particularly to differentiate tuberculomas from high grade gliomas. Differentiating these two lesions was challenging as Choline increase was seen in all tuberculomas. Only lesions with Cho:Cr and Cho:NAA ratios <2.00 could be confidently diagnosed as tuberculomas. This resulted in only a modest 51.6% improvement in diagnostic performance of single-voxel MRS for this indication. Multi-voxel MRS theoretically has the potential to greatly improve the ability to differentiate between these two lesions.

MRS is valuable in differentiating between low grade gliomas and cerebral infarcts based on observing increased spectra of Choline and Myo-inositol in low grade gliomas. The differences seen were not statistically significant due to low frequencies.

MRS is valuable in differentiating between recurrent tumour and radiation necrosis based on observing increased spectra of Choline in recurrent tumours, along with increased Cho:NAA ratios and decreased NAA:Cr and NAA:Cho ratios. Once again, the differences seen were not statistically significant due to low frequencies.

14. Recommendations

- A local study to compare the diagnosis obtained from MRI+MRS against histopathological diagnosis to determine sensitivity and specificity of brain MRS in our local setup.
- A local study with a larger sample size designed to study the efficacy of brain MRS targeted to particular indications such as cerebral infarcts vs. low grade gliomas, and recurrent tumour vs. radiation necrosis.
- A local study to compare Myo-inositol levels between low grade and high grade gliomas.
- Introduction of Multi-voxel MRS (Chemical Shift Imaging) to improve the differentiation of tuberculomas from high grade gliomas, since they form the largest number of indications for MRS; and since Single-voxel MRS has shown limitations.
- Performing brain MRS as part of a complete MRI examination for any intracranial mass lesion where MRI alone is inconclusive, and the indication is appropriate as deemed by the radiologist; preferably at no additional cost to the patient; and at the same sitting as the initial MRI examination. Increasing the number of MRS examinations performed will greatly help bring down the cost to the patient.

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Appendix A. Timeline of Research Study

A Gantt chart illustrating the timeline of the research study is presented in (**Figure 39**). The table below provides the details which were used to create the Gantt chart.

Table 21: Task assignments, durations, start and end dates, % completed and predecessors for dependencies for the research study, which were used to create the Gantt chart presented in **Figure 39**.

Outline	Task Name	Start Date	End Date	Predecessors	Completed (%)	Duration (Wk.Days)
1	Preliminary Literature Search	30/07/12	26/09/12		100	43
2	Research Question Development	03/09/12	01/10/12		100	21
3	Methodology Development	03/09/12	01/10/12		100	21
4	Proposal Writing	03/09/12	15/10/12		100	31
5	Approval by Supervisors	16/10/12	09/11/12	4	100	19
6	ERC Review of Draft Proposal	12/11/12	14/01/13	5	100	46
7	Proposal Corrections based on ERC Recommendations	15/01/13	11/03/13	6	100	40
8	ERC Approval of Final Proposal	12/03/13	15/04/13	7	100	25
9	Data Collection	16/04/13	09/10/13	8	100	127
10	Data Analysis	10/10/13	28/11/13	9	100	36
11	Write-up of Draft Report	02/12/13	10/01/14	10	100	30
12	Approval by Supervisors	13/01/14	31/01/14	11	0	15
13	Production of Final Study Report	03/02/14	21/02/14	12	0	15

Gantt Chart Illustrating Study Timeline

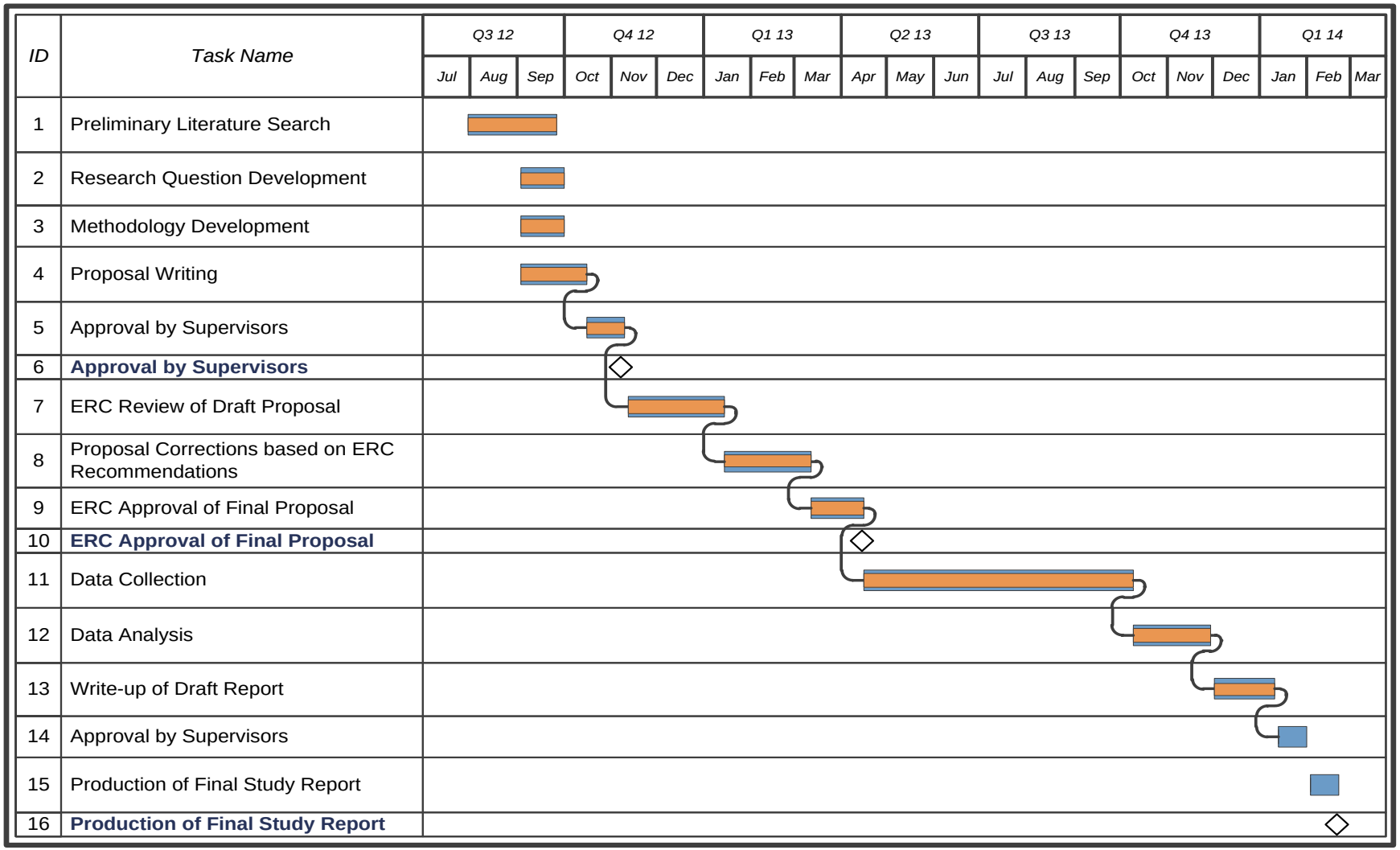

Figure 39: Gantt chart illustrating study timeline. = Completed, = Incomplete, = Dependencies, ◆ = Milestones

Appendix B. Common Chemical Shift Assignments in Neurology

Table 22: Common Chemical Shift Assignments in Neurology(41)

Metabolite	Characteristic Chemical Shift (ppm)	Normal Function	Increased	Decreased
Lipids (Lip)	0.9 & 1.7	Cell membrane component, abundant in scalp/skull	Hypoxia, trauma, expanding neoplasia, lymphoma, PML, toxo, crypto, SBS, inflammatory disease	
Lactate (Lac)	1.3	Product of anaerobic glycolysis	Hypoxia anoxia, near-drowning, ICH, stroke, hypo- and hyperventilation, IBEM, leukodystrophies, hydrocephalus, lymphoma (lac + lipid), PML toxo, trauma, neoplasia, necrosis, cyst	
Alanine (Ala)	1.5	Non-essential amino acid	Meningioma, ischemia	
Acetate (Ace)	1.9	Important anabolic precursor	Neoplasia, ischemia, abscess, normal neonate	
***N*-acetyl aspartate (NAA)**	2	Abundant CNS amino acid; osmolyte; GM neuronal marker; WM axonal density marker	Normal infant, recovered MS, hyperosmolar state, trauma, Canavan's disease	Neuronal loss, stroke, dementia, developmental delay, hypoxia, anoxia, ischemia, AD, other dementias, ICH, near-drowning, MS, epilepsy, neoplasia, DM, trauma, lymphoma, PML, leukodystrophies, toxo, crypto, encephalitis (herpetic), hyponatremia, SIADH
Glutamate (Glu)	2.0-2.4	Major excitatory neurotransmitter	Hypoxia, ischemia, near-drowning, HE, developmental delay	Hyponatremia, SIADH, trauma, AD
Glutamine (Gln)	2.1-2.5	Glutamate precursor; astrocyte marker	Hypoxia, ischemia, near-drowning, HE, developmental delay	Hyponatremia, SIADH, trauma, AD

Metabolite	Characteristic Chemical Shift (ppm)	Normal Function	Increased	Decreased
γ-Aminobutyric acid (GABA)	1.9, 2.3, 3.0	Major inhibitory neurotransmitter	Used to monitor treatment of seizures or muscle spasms	
Succinate (Suc)	2.4	Part of TCA cycle	Brain abscess	
Phenylalanine (Phe)	3.1 & 3.3	Catecholamine precursor	PKU	
Creatine (Cr)	3.03	Cell energy marker, includes phosphocreatine	Trauma, hyperosmolar, age	Stroke, hypoxia, PML, neoplasia, lymphoma, hypoxia, SIADH, hyponatremia, normal infant, trauma, toxo, crypto
Choline (Cho)	3.19	Complex of membrane markers	Neoplasia, ischemia, stroke, trauma, AD, MS, inflammation, nonspecific brain injury, normal neonate, normal elderly, post liver transplant, hyperosmolar state, PML	Asymptomatic liver disease, HE, stroke (rarely), dementias, toxo, crypto, hyponatremia, SIADH
Taurine (Tau)	3.3-3.4	Osmoregulation; modulates action of neurotransmitters	Neonate, diet	
***Scyllo*-inositol (s-Ins)**	3.34	isomer of inositol	? AD	HE
Glucose (Glc)	3.4-3.9	Essential brain nutrient; can increase m-Ins peak	DM	
***Myo*-inositol (m-Ins)**	3.5 & 3.6	Astrocyte marker; cerebral osmolyte	AD, renal failure, DM, recovered hypoxia, hyperosmolar states, PML	Chronic HE, hypoxia, stroke neoplasia, lymphoma, toxo, crypto, hyponatremia; SIADH

Leukodystrophies refer to Canavan's disease and Alexander's disease.

AD = Alzheimer's disease; CNS = central nervous system; Crypto = cryptococcoma; DM = diabetes mellitus, GM = grey matter; HE = hepatic encephalopathy, IBEM = inborn error of metabolism; ICH = intracranial haemorrhage; MS = multiple sclerosis; PKU = phenylketonuria; PML = progressive multifocal leukoencephalopathy; SBS = shaken baby syndrome; SIADH = syndrome of inappropriate antidiuretic hormone; TCA = tricarboxylic acid; Toxo = toxoplasmosis; WM = white matter.

Appendix C. Consent Information Document

Introduction

My name is Dr. Mufaddal N. Wajihi. I am a postgraduate student at the department of Diagnostic Imaging and Radiation Medicine, University of Nairobi. As part of my training, I am required to undertake a research study. I am conducting a study to document the use of **Magnetic Resonance Spectroscopy (MRS) in the diagnosis of mass lesions in the brain**.

Your doctor referred you for an MRS (Magnetic Resonance Spectroscopy) examination, to help diagnose your illness. MRS is a special type of MRI (Magnetic Resonance Imaging) that is very safe.

Before you agree to undergo the MRI/MRS examination, it is important that you understand what MRI is, what its benefits are, how safe it is, what its contraindications are, and what adverse effects you may encounter. You will be required to fill out the MRI safety questionnaire, for the radiologist to decide whether it is safe for you to undergo the MRI scan.

What is MRI?

- MRI scanner uses a very strong magnet and radio waves, to create exceptionally detailed images of the inside of your body, especially soft tissues like the brain. This is very beneficial in diagnosing diseases where normal X-ray and CT scans are inadequate. Unlike X-ray and CT scan, MRI does not use harmful radiation. The scan takes approximately 20-30 minutes. The magnetic field has no harmful effects on the body, and is very safe. Many studies have proven this.
- MRI uses a strong magnet, so all metallic objects must be removed before you enter the MRI room. You need to correctly fill the MRI safety questionnaire to ensure that no metallic object is within your body, such as cardiac pacemakers, cochlear implants or bone plates, screws or rods. This may prohibit you from having the MRI scan.
- The scan takes place while you lay still in a confined space for approximately 30 minutes. If you are claustrophobic or feel uneasy or anxious about this, you need to let the radiographer know in advance. Once the scanning is underway, you will hear a vibrating sound. You will be provided with headphones or ear plugs to minimise the noise. You need to remain still during the scan so that the quality of images is not spoilt. You will be in constant communication with the radiographer during this time.

Information for Female Patients

- If you are pregnant, or think you might be pregnant; you need to inform the radiographer, so that the radiologist can discuss your situation with yourself and your referring doctor.
- MRI scans are normally not done in the first trimester, unless the radiologist and your doctor feel it is absolutely necessary, and the benefit outweighs the risk, and that no alternative suitable test is available.

MRI Contrast (Dye)

- During your scan, you may require an intravenous injection of a contrast dye known as Gadolinium. The benefit of the contrast agent is immense, in that it improves the accuracy of the diagnosis, and helps visualize certain diseases which would otherwise be missed.

- The contrast is administered through a tiny plastic tube called a cannula, which is inserted into a vein in your arm. This procedure is done by a trained and experienced person. You may feel some minor pain or discomfort during this procedure.
- In most cases, the injection of contrast is very safe, and no side effects are felt. A minority (1 in 3,500) of patients may experience minor side effects like headache, nausea, sneezing or hives; which settles very fast. It can be easily treated with medicine if necessary.
- Very rarely (1 in 1 million), a severe anaphylactic reaction may occur, such as difficulty breathing, difficulty swallowing, fast heart rate, or shock. This requires emergency resuscitation. Despite this, a patient may die, although fortunately it is extremely rare (1 in 10 million).
- If you have kidney (renal) impairment or failure, you should indicate this in the safety questionnaire and inform the radiographer. You should NOT get a Gadolinium injection, because of the risk of life threatening NSF (Nephrogenic Systemic Fibrosis).
- If you have had a contrast reaction previously to an X-ray, CT or Angiography procedure, you should indicate this in the safety questionnaire and inform the radiographer, because you are at higher risk of contrast reaction.
- If you are a breastfeeding mother, it is safe for you to receive a Gadolinium contrast injection and continue breastfeeding. Only 0.0004% of the dose is absorbed by the child, which has no known adverse effects. If you have concerns, you may abstain from breastfeeding for 24 hours.

Additional information for parents / guardians of patients who are minors

- You will be required to complete the MRI safety questionnaire on behalf of the patient, and provide consent accordingly.
- You may accompany the patient into the MRI room after complying with safety instructions from the radiographer.
- It is important you help the child remain calm and relaxed, and ensure the child remains still for the duration of the scan.
- If you are a woman who is pregnant or think you may be pregnant, you should not enter the MRI room. You should bring another suitable adult who can stay with the child.
- The patient will only be included in the study if you **consent**. You can refuse to allow your child to be included in the study if you want. The patient will have the opportunity to provide **assent** to participate in the study, or to refuse if he/she so wishes.

About the investigator

- My name is Dr. Mufaddal N. Wajihi. I am a postgraduate student at the University of Nairobi. I am studying Radiology. I am required to carry out a research project as part of my academic requirements.

About the study

- The purpose of this research is to study the benefit of MRS added to MRI in diagnosis of certain brain diseases.
- You were referred by your doctor with a request for MRS to be done. Therefore I would like to recruit you into my study.

- MRS (Magnetic Resonance Spectroscopy) is a safe, non-invasive additional test which can be done at the same time as MRI, using a normal MRI scanner. It only requires you to spend an extra 10 minutes inside the MRI scanner, so that specialized software can perform an advanced analysis of the disease in your brain.
- It provides additional information which is very useful in allowing the radiologist to make a more accurate diagnosis of your disease.
- It is just as safe as normal MRI. It has no additional risks or contraindications to those that have been described above.

Participation in the study

- Participation in this study is entirely voluntary. You are not being forced to participate in the study. You have the right to refuse to participate in the study without giving any reason, and without your medical care or legal rights being affected.
- If you decide not to participate in the study, your MRS test will still be done, (as long as you have consented and met MRI safety requirements). You will not be denied the scan which your doctor has requested. You and your doctor will still get the results of your scan, whether or not you participate in the study.
- If you do agree to participate in the study, your name and personal information will NOT be included in the study report and will be kept confidential at all times. Forms used to collect data will be destroyed at the end of the study.
- You will not receive any compensation, either financially or otherwise, for participating in this study. You will still be required to meet the cost of the examination as requested by your doctor, and as agreed upon by the diagnostic imaging service provider.
- You will not receive any extra advantage or benefit by participating in the study.
- You will not receive any gift or reward for participating in the study.
- You will receive the same standard of care that all other patients receive, regardless of whether or not you participate in the study.
- The outcome of this study will be used for academic purposes only. The information it provides will increase our knowledge of brain diseases, and may help improve the diagnosis of certain brain diseases.

Confidentiality of Information

- All data collected for the research study will be kept confidential. Information may be shared by the researcher with faculty supervisors, solely to facilitate the research. Data which is presented in the final report will not include your name or any personal information.
- All forms used to collect data will be treated with utmost confidence during the research period. All such material will be destroyed once the research study is completed.

Appendix D. MRI Safety Questionnaire and Consent Form

Patient's Name: _____ Date: _____

Sex: _____ Age: _____ DOB: _____ Weight: _____

1. Have you had any previous surgery? List all with dates.

Yes ☐ No ☐

_____

_____

2. Do you have any of the following implants / devices in or on your body?

Cardiac Pacemaker?	Yes ☐	No ☐
Implanted Cardiac Defibrillator?	Yes ☐	No ☐
Artificial heart valve?	Yes ☐	No ☐
Aneurysmal clips?	Yes ☐	No ☐
Shunt, Filter, Coil or Stent?	Yes ☐	No ☐
Neurostimulator or Bone growth stimulator?	Yes ☐	No ☐
Implanted drug pumps?	Yes ☐	No ☐
Any access ports or catheters?	Yes ☐	No ☐
Cochlear (ear) or Ocular (eye) implant?	Yes ☐	No ☐
Wire sutures or surgical staples?	Yes ☐	No ☐
Have you ever had metal in your eyes or worked extensively with metal?	Yes ☐	No ☐
Dentures, dental plates or any dental work?	Yes ☐	No ☐
Joint replacement?	Yes ☐	No ☐
Bone/joint pin, screw, nail, wire or plate?	Yes ☐	No ☐
Hearing Aid?	Yes ☐	No ☐
Bullet, shrapnel or any metal piece in the body?	Yes ☐	No ☐
Body piercings (internal or external)?	Yes ☐	No ☐
Tattoos?	Yes ☐	No ☐

3. For females of reproductive age

Are you pregnant?	Yes ☐	No ☐
If unsure, is it possible you may be pregnant?	Yes ☐	No ☐

When was your last menstrual period? _____

4. Contrast study questionnaire

Have you ever had a radiological test where contrast or dye was used?	Yes ☐	No ☐

Did you have any reaction during or after receiving the contrast or dye? Yes ☐ No ☐

If yes, provide details.

_____

_____

Do you have any allergies? Yes ☐ No ☐

If yes, provide details.

_____

_____

Do you suffer from kidney (renal) disease? Yes ☐ No ☐

Are you on any medication? Please list. Yes ☐ No ☐

_____

_____

5. *For females of reproductive age*

Are you breast-feeding? Yes ☐ No ☐

Before you enter the MRI room, you must remove all metallic objects on your person, including mobile phone, watch, coins, credit cards, keys, jewellery, body piercings, hair clips and extensions, dentures, hearing aids, eyeliner, prosthetic limbs, and any object with metallic component.

Patient consent to MRI procedure and intravenous administration of MRI contrast. Please Tick. *(This is only consent for the MRI/MRS procedure, not for participation in the research study.)*

- ☐ I confirm that the information provided above is correct to the best of my knowledge.
- ☐ I have read the information provided in the Consent Information Document, and I am fully aware of the benefits and risks of having an MRI done. I am also aware of the benefits and risks of being given an injection of the gadolinium MRI contrast.
- ☐ I have had the opportunity to ask questions, which have been adequately answered.
- ☐ I thereby give consent to proceed with the MRI examination, and consent to the injection of intravenous contrast.
- ☐ I understand that I can consent to the MRI/MRS and still refuse to take part in the study.

Patient Name and Signature: _____

Parent or Guardian Name and Signature (if patient is a minor) – (State relationship):

_____

Emergency contact: _____

Witnessed by (Name and Signature): _____

Appendix E. Consent Form – To Participate in Research Study

Title of Study: Clinical Application of Magnetic Resonance Spectroscopy in Diagnosis of Intracranial Mass Lesions.

Name of Researcher: Dr. Mufaddal N. Wajihi, M.B.Ch.B.
Postgraduate Radiology Resident
University of Nairobi, Department of Diagnostic Imaging and Radiation Medicine

☐ I hereby confirm that I have read and understood the **Consent Information Document** provided for the above study. I have been given the opportunity to ask questions which have been adequately answered

☐ I understand that my participation is voluntary and that I have not been forced to participate. I understand that I can decline without giving any reason, without my medical care or legal rights being affected.

☐ I understand that I will not receive any compensation either financial or otherwise, and will not receive any preferential treatment, gift or reward, for participating in the above study.

☐ I understand that my personal information will be kept confidential, but that any relevant medical information regarding the results of my scans and the data collected will be accessible to the researcher, and may be looked at by his supervisors where relevant to the study. I give them permission to have access to this information.

☐ **I hereby consent to take part in the above study.**

Name of Participant:	Date:	Signature:
_____	_____	_____

Contact (Mobile Number): _____

Patient ID/MRI No. _____

Name of Parent/Guardian providing consent for patient who is a minor	Date:	Signature:
_____	_____	_____

Name of Person taking consent:	Date:	Signature:
_____	_____	_____

Name of Witness:	Date:	Signature:
_____	_____	_____

Appendix F. Fomu ya Idhini – Ili Kushiriki katika Utafiti

Kichwa cha Utafiti: Utumizi wa MRS (*Magnetic Resonance Spectroscopy*) Katika Utambuzi wa Vidonda vya Ubongo.

Jina la Mtafiti: Dr. Mufaddal N. Wajihi, M.B.Ch.B.
Mwanafunzi Wa Shahada Ya Uzamili Katika *Radiology*
Chuo Kikuu cha Nairobi, Idara ya *Radiology*

☐ Mimi hili huthibitisha kuwa nimesoma na kuelewa (au nimekuwa nikielezewa) hati ya ***"Consent Information Document"*** kwa ajili ya utafiti huu. Nimepewa fursa ya kuuliza maswali ambayo majibu yao yamekuwa ya kutosha.

☐ Ninaelewa kwamba ushiriki wangu ni wa hiari na kwamba sijalazimishwa kushiriki. Naelewa kwamba naweza kukataa bila ya kutoa sababu yoyote, bila ya huduma ya matibabu yangu au haki za kisheria kuathirika.

☐ Ninaelewa kwamba sitapokea fidia yoyote, fedha au vinginevyo, na sitapokea matibabu yoyote ya upendeleo, zawadi au tuzo, kwa ajili ya kushiriki katika utafiti huu.

☐ Ninaelewa kwamba taarifa yangu binafsi itakuwa siri, lakini kwamba yoyote husika matibabu, habari kuhusu matokeo ya uchunguzi wangu na taarifa zilizokusanywa itakuwa itapatikana kwa mtafiti, na inaweza kuangaliwa na wasimamizi wake. Mimi nawapa ruhusa ya kuwa na upatikanaji wa habari hii.

☐ **Ninatoa idhini ya kushiriki katika utafiti huu.**

Jina la Mshiriki: _____ Tarehe: _____ Sahihi: _____

Nambari Ya Simu Kwa Kuwasiliana: _____

Nambari ya Utambulisho ya Mshiriki: _____

Jina la Mzazi / Mlezi anayetoa Idhini kwa Mshiriki ambaye ni Mdogo. _____ Tarehe: _____ Sahihi: _____

Jina la Mtu anayechukua Idhini: _____ Tarehe: _____ Sahihi: _____

Jina la Shahidi: _____ Tarehe: _____ Sahihi: _____

Appendix G. Assent Form – For Minors to Participate in Research Study

Title of Study: Clinical Application of Magnetic Resonance Spectroscopy in Diagnosis of Intracranial Mass Lesions.

Name of Researcher: Dr. Mufaddal N. Wajihi, M.B.Ch.B.
Postgraduate Radiology Resident
University of Nairobi, Department of Diagnostic Imaging and Radiation Medicine

Who am I?

My name is Mufaddal Wajihi. I am a doctor. I am studying to become a radiologist. A radiologist is a doctor who specializes in reading and understanding pictures of the inside of your body.

What is a research study?

A research study is when someone collects a lot of information to learn more about something. Doctors who do research are also called researchers.

Why am I doing this study?

I am doing this study to be able to understand how certain sicknesses of the brain appear on a special MRI scan, and how it helps us getting to know better what is wrong with the brain.

What will happen if you take part in my study?

If you agree to take part in the study, you will be asked to lie in a machine which takes very nice pictures of the inside of your brain so that we can tell what sickness it has. The machine is safe, but it has a small space, and makes lots of noise. To take pictures of your brain, you must wear a helmet and ear plugs and lay very still. You may be given an injection which will help us see better where the problem is. This may cause some small pain for a short while. Once the scan is complete, I will have access to your pictures and results, so that I can compare them with others like yours.

Why do I want you to take part in my study?

Your doctor has asked for this scan to be done, so that he can know why you are sick. I want you to take part in the study so that I can learn more about the sickness you have, how it looks on the pictures of your brain; so that I can compare it together with pictures of other people who are sick like you.

Will my information be made public?

No, not at all. All information collected regarding your scans will be kept private. Your name will not be mentioned in the final report. All records with personal information will be destroyed once the research study is completed.

Do you have to have to take part in the study?

Taking part in the study is YOUR choice. You do not have to be in the study. Even if your parents/guardians say it is OK for you to be in the study, you can still say NO. You can say yes now and then change your mind at any time.

Will anything bad happen if you take part in the study?

The MRI scan is very safe. Your parents/guardians and the doctors will make sure it is safe for you to have the scan done. You may feel anxious because you will be in a small space. You may need to have an injection which may cause some pain, and may cause some side effects. There is a document (**Consent Information Document**) which you can read, or will be explained to you which explains all the benefits and risks of having an MRI scan. But there are trained doctors who will ensure you are safe at all times. Your parent/guardian can be with you during the scan.

Will you get a reward for being in the study?

You will NOT get any gift or reward for taking part in the study. But by taking part, you will help us get more knowledge about your sickness.

What will happen if you say NO to take part in the study?

You can refuse to take part in the study if you want. You will not be punished or victimized. Your test will still be done as your doctor has asked, as long as your parent/guardian has approved and it is safe for you to have the scan done. The only difference is that your results will not be included my study.

You can ask any questions you may have about this study or the scan.

Assent from child / patient who is a minor:

☐ I have read (or have had read to me) the contents of this assent form and have been encouraged to ask questions. I have received answers to my questions. I agree to take part in this study.

Write your name here if you agree to be in the study:

_____

Sign here if you agree to be in the study: _____ Date: _____

Name and Signature of Person Obtaining Assent:

_____ Date: _____

Name and Signature of Witness: _____ Date: _____

Appendix H. Fomu ya Kukubaliana – Kwa Watoto Kushiriki katika Utafiti

Kichwa cha Utafiti: Utumizi wa MRS (*Magnetic Resonance Spectroscopy*) Katika Utambuzi wa Vidonda vya Ubongo.

Jina la Mtafiti: Dr. Mufaddal N. Wajihi, M.B.Ch.B.
Mwanafunzi Wa Shahada Ya Uzamili Katika *Radiology*
Chuo Kikuu cha Nairobi, Idara ya *Radiology*

Mimi ni nani?

Jina langu ni Mufaddal Wajihi. Mimi ni daktari. Ninasoma kuwa *radiologist*. *Radiologist* ni daktari ambaye ni mtaalamu katika kusoma na kuelewa picha za ndani za mwili wako.

Utafiti ni nini?

Utafiti ni wakati mtu anakusanya habari nyingi ya kujifunza zaidi kuhusu kitu. Madaktari ambao wanaofanya utafiti wanaitwa watafiti.

Kwa nini ninafanya utafiti huu?

Ninafanya utafiti huu ili kuwa na uwezo wa kuelewa jinsi baadhi ya magonjwa ya ubongo yaonekana kwenye uchunguzi maalum yanayoitwa *MRI*, na jinsi inatusaidia kupata kujua bora nini ni vibaya kwa ubongo.

Nini kitatokea kama wewe utashiriki katika utafiti wangu?

Kama unakubali kushiriki katika utafiti, utaulizwa kulala ndani ya mashine ambayo inachukua picha nzuri sana ya ndani ya ubongo wako ili tuweze kuwaambia nini ugonjwa unao. Mashine ni salama, lakini ina nafasi ndogo, na hufanya kelele mengi. Ili kuchukua picha za ubongo wako, lazima uvae aina ya helmeti na kifaa kufunika masikio yako na ulale bila kusonga. Unaweza pewa sindano ya dawa ambayo itatusaidia kuona vizuri pahali tatizo ndipo. Hii inaweza kusababisha maumivu kidogo kwa muda mfupi. Mara uchunguzi umekamilika, nitapata matokeo yako, ili niweze kulinganisha na zile za wagonjwa wengine.

Kwa nini ninataka wewe kushiriki katika utafiti wangu?

Daktari wako ameomba ufanyiwe uchunguzi huu, ili aweze kujua ugonjwa wako. Ninataka wewe kushiriki katika utafiti ili niweze kujifunza zaidi kuhusu ugonjwa wako, na jinsi inaonekana katika picha za *MRI*; ili niweze kulinganisha pamoja na picha za watu wengine ambao ni wagonjwa kama wewe.

Je, maelezo yangu yatakuwa wazi kwa wananchi?

Hapana. Habari zote zilizokusanywa kuhusu uchunguzi yako zitakuwa siri. Jina lako halitatajwa katika ripoti ya mwisho. Kumbukumbu zote na habari za kibinafsi itaharibiwa mara moja utafiti unapokamilika.

Je, ni lazima kushiriki katika utafiti?

Kushiriki katika utafiti ni chaguo lako. Hata kama wazazi wako / walezi wasema ni SAWA kwa wewe kuwa katika utafiti, bado unaweza kusema HAPANA. Unaweza sema ndiyo sasa, na kisha ubadilishe mawazo yako wakati wowote.

Je, kuna kitu mbaya ambacho kitatokea kama wewe hushiriki katika utafiti?

Uchunguzi wa *MRI* ni salama sana. Wazazi / walezi wako na madaktari watahakikisha kuwa ni salama kwa wewe kufanyiwa uchunguzi huu. Unaweza kuhisi wasiwasi kwa sababu utakuwa katika nafasi ndogo. Unaweza pewa

sindano ya dawa ambayo inaweza kusababisha maumivu kidogo, na madhara. Kuna hati (*"Consent Information Document"*) ambayo unaweza soma, au inaweza fafanuliwa kwako, ambayo inaeleza wote faida na hatari ya kufanyiwa uchunguzi wa *MRI*. Lakini kuna madaktari wenye ujuzi ambao watahakikisha kuwa uko salama wakati wote. Mzazi / mlezi wako anaweza kuwa na wewe wakati wa uchunguzi.

Je, utapata tuzo kwa ajili ya kushiriki katika utafiti?

Wewe hutapata zawadi yoyote au malipo kwa ajili ya kushiriki katika utafiti. Lakini ukishiriki, utatusaidia kupata maarifa zaidi kuhusu ugonjwa wako.

Nini kitatokea kama wewe hukataa kushiriki katika utafiti?

Unaweza kukataa kuchukua sehemu katika utafiti kama unataka. Huwezi adhibiwa. Uchunguzi wako bado utafanyika kama daktari wako amesema, basi tu mzazi / mlezi wako amekubali na ni salama kwako kufanyiwa uchunguzi. Tofauti tu ni kwamba matokeo yako hayatahusishwa kwenye utafiti wangu.

Unaweza kuuliza maswali yoyote kuhusu utafiti huu au uchunguzi wa *MRI*.

Kukubaliana kutoka mshiriki ambaye ni mdogo:

☐ Nimesoma (au nimefafanuliwa) yaliyomo katika fomu hii ya kukubaliana na nimehamasishwa kuuliza maswali. Nimepokea majibu ya maswali yangu. **Ninakubaliana na kushiriki katika utafiti huu**.

Andika jina lako hapa kama unakubali kuwa katika utafiti:

_____

Chora sahihi (saini) hapa kama unakubali kuwa katika utafiti:

_____ Tarehe: _____

Jina na Sahihi la Mtu Anayepokea Ruhusa kutoka Mtoto:

_____ Tarehe: _____

Jina na Sahihi ya Shahidi: _____ Tarehe: _____

Appendix I. Data Collection Form

A.			
Title of Study:	Clinical Application of Magnetic Resonance Spectroscopy in Diagnosis of Intracranial Mass Lesions		
Investigator:	Dr. Mufaddal Wajihi (M.B.Ch.B.), Postgraduate Student, Dept. of Diagnostic Imaging and Radiation Medicine, University of Nairobi		
Patient ID:	MRS Number:	Age:	Sex:

B.		
MRI Diagnosis: (if only single diagnosis)	☐ Low grade glioma ☐ High grade glioma ☐ Lymphoma ☐ Metastasis ☐ Meningioma ☐ Schwannoma	☐ Cerebral infarct ☐ Pyogenic Abscess ☐ Tuberculoma ☐ Toxoplasmosis ☐ Recurrent tumour ☐ Radiation necrosis
	☐ Other:	
MRI Diagnosis: (if multiple differentials)	☐ No single diagnosis	

C.

Indication for MRS:

☐ Low or high grade glioma.
☐ Metastasis or high grade glioma.
☐ Low-grade glioma or infarction.
☐ Brain tumour or abscess.
☐ Recurrent tumour or radiation necrosis.
☐ Other: _____

D. MRS Findings: Short TE (31 msec)

Metabolite	***Pos./ppm***	***Height***	***Ht/Cr***	***Area***	***Ar/Cr***
Lip					
Lip/Lac					
NAA					
Cr					
Cho					
Mi					
Other					
Other					

MRS Findings: Intermediate TE (144 msec)					
Metabolite	***Pos./ppm***	***Height***	***Ht/Cr***	***Area***	***Ar/Cr***
Lip					
Lip/Lac					
NAA					
Cr					
Cho					
Mi					
Other					
Other					

Metabolite Ratios:

	NAA/Cr	**NAA/Cr (h)**	**Cho/Cr**	**Cho/Cr (h)**	**NAA/Cho**	**NAA/Cho (h)**	**Cho/NAA**	**Cho/NAA (h)**
31ms								
144ms								

Observed MRS Spectrum Appearance: (— / ↑ / ↓) "—" = no change, "↑" = increased, "↓" = decreased

NAA	**Cr**	**Cho**	**Lip/Lac**	**Mi**	**Glx / Other**

MRS Diagnosis: (if only single diagnosis)	☐ Low grade glioma ☐ High grade glioma ☐ Lymphoma ☐ Metastasis ☐ Meningioma ☐ Schwannoma	☐ Cerebral infarct ☐ Pyogenic Abscess ☐ Tuberculoma ☐ Toxoplasmosis ☐ Recurrent tumour ☐ Radiation necrosis
	☐ Other:	
MRS Diagnosis: (if multiple differentials)	☐ No single diagnosis	

Did MRS improve the imaging diagnosis? ☐ Yes ☐ No

Comments/Remarks:

Appendix J. Budget

	Allocation	Breakdown	Amount in KSh
1.	Stationery	Printing paper @1000/- Biro pens (1 box)@1000/- Folders @200	3,000 1,000 2,000
2.	Ethics board	Ethics fees	2,000
3.	Secretarial services	Typist fees Photocopy	3,000 4,000
4.	Data collection and analysis	Statistician services	25,000
5.	Image acquisition	Scanning of images Digital transfer of images	3,000 2,000
6.	Printing and binding	Drafts Proposal Final report	4,000 9,000 12,000
	TOTAL AMOUNT		**70,000**