

*East African Medical Journal Vol. 88 No. 3 March 2011*

## SINGLE VOXEL MAGNETIC RESONANCE SPECTROSCOPY IN DISTINGUISHING FOCAL NEOPLASTIC FROM NON-NEOPLASTIC BRAIN LESIONS

J. Sande, MBChB, MMed (Rad), J. Masesa, MBChB, MMed (Rad), Department of Radiology and Diagnostic Imaging, J. Jowi, MBChB, MMed (Neuro), Ass. Professor, Department of Medicine, Z. Ali, MBChB, MMed (Rad), Department of Radiology and Diagnostic Imaging, Aga Khan University, Nairobi, P. O. Box 30270-00100, Nairobi, Kenya

Request for reprints to: Dr. J. Sande, Department of Radiology and Diagnostic Imaging, Aga Khan, University, P.O. Box 63928- 00619 Nairobi, Kenya

## SINGLE VOXEL MAGNETIC RESONANCE SPECTROSCOPY IN DISTINGUISHING FOCAL NEOPLASTIC FROM NON-NEOPLASTIC BRAIN LESIONS

J. SANDE, J. MASESA, J. JOWI and Z. ALI

### ABSTRACT

**Objective:** Assess diagnostic utility of combined magnetic resonance imaging and magnetic resonance spectroscopy (MRI, MRS) in differentiating focal neoplastic lesions from focal non- neoplastic (infective or degenerative) brain lesions.

**Design:** Descriptive, analytical - prospective study.

**Setting:** The Aga Khan University MRI department.

**Subject:** Seventy four consecutive patients.

**Main outcome measures:** Kappa measurement of agreement was used to determine the agreement between MRI and MRI, MRS with the final diagnosis. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the two tests were calculated. The difference between the number of indeterminate lesions in the two tests was determined. Logistic regression demonstrated the role of confounding factors in the diagnostic use of MRS.

**Results:** MRI, MRS had a higher agreement with the final diagnosis than MRI in isolation. The sensitivity of MRI, MRS was 4.82 times greater than that of MRI. MRI, MRS had a 1.7% increase in accuracy. MRI, MRS reduced the indeterminate MRI lesions by 5.4%. Logistic regression showed that for lesions which were enhancing, MRS yield was more helpful if the voxel position included the enhancing part.

**Conclusion:** MRI, MRS is better than MRI alone in characterisation of neoplastic from non- neoplastic focal brain lesions.

### INTRODUCTION

Intracranial pathology presents several imaging challenges including the differentiation between various causes of disease, even when classified broadly into infective, degenerative and/ or malignant(1).

Conventional magnetic resonance imaging (MRI) provides structural cross-sectional images that depict anatomical detail. Most patients need further work-up such as biopsy or surgery to classify a lesion (2 - 4).

Functional/advanced MRI techniques allow insight into such processes as, the freedom of water molecule movement, micro-vascular integrity, haemodynamic characteristics, and the molecular makeup of certain compounds of masses (5). These increase the diagnostic yield of MRI. This study focused on magnetic resonance spectroscopy (MRS).

The technical aspect of MRS involves selecting a

volume of tissue referred to as a voxel. From the voxel, MRS generates a signal which corresponds to the different molecules in the tissue, and their respective quantities. This signal can be referred to as a signature. This characteristic can be used to diagnose certain metabolic disorders and to provide information on tumor metabolism.

MRS may prove to be of benefit in the diagnosis of focal brain lesions located in anatomically unfavorable sites for biopsy (6). This includes many childhood focal brain tumors, whose diagnosis can be complicated because of their frequent adjacent location to crucial structures (7).

So far, studies have shown that advanced MR is the imaging modality of choice for the characterisation of various intracranial lesions (8,9).

The use of single voxel spectroscopy involves interpreting a signal/ signature which consists of absolute values and ratios of metabolites. Various

patterns have been attributed to certain disease entities, but this field is still under investigation and no definite associations have been published. Spectroscopy is not used by itself to make a diagnosis but rather, its appearance is interpreted with the corresponding MRI findings.

Some aspects of the added advantage of MRS have been published(8, 9). Its use has been advocated in the division of malignant tumors into high or low grade (6); determining extent of malignant lesions beyond that which is gross; indicating the difference between tumor recurrence and post radiation changes (10,11), to mention a few.

The full untapped potential of MRS is yet to be realised. Literature has not focused on the benefit of MRS in the clinical differentiation of neoplastic from non- neoplastic (infectious or degenerative) focal brain lesions.

## MATERIALS AND METHODS

*Study design:* A prospective, descriptive and analytical study.

*Study population:* Patients referred to the MRI department of Aga Khan University Hospital, Nairobi (AKUH), for an MRI examination of the brain.

*Study procedures:* Conventional multisequential (T1, T2, fluid attenuated inversion recovery, diffusion, gradient echo, pre and post contrast imaging) MRI scanning was done. The contrast used was gadolinium gadopentetic acid, dimeglumine salt at a dose of 0.2ml/ kg body weight given via hand injection. The images were viewed in three planes that is axial, sagittal and coronal. If a focal lesion was seen, the MRI technologist who has a greater than ten year experience in MRI imaging, resident radiologist and MRI consultant radiologist, working as a team, would agree on whether or not MRS would be added.

*Inclusion criteria:* All consecutive patients with a well defined lesion seen on at least two different sequences. Informed consent was taken.

*Exclusion criteria:* Patients in whom contrast was not administered. This included patients with severely impaired renal function because of the risk of nephrogenic systemic sclerosis (NSF), pregnant and lactating women (16). This exclusion criterion was because the administration of contrast had a direct bearing on MRS voxel positioning (17).

*Imaging:* This was done with a 1.5T clinical scanner and head coil(18). The MRI and MRI, MRS protocols employed were based on current guidelines set out by General Electronic Healthcare for imaging focal brain lesions (19). To perform MRS a volume

of tissue was selected after the conventional post contrast image was acquired. In order to maximise the yield of the MRS spectrum, various precautions were followed. Studies have shown that the voxel should include most, if any of the solid-appearing parts of the tumors to minimize any partial volume averaging from surrounding fluid. Regions of interest did not include any significant fluid, which can also reduce the MRS spectral quality (13). Adjustment of voxel size depended on the lesion size, the former being optimised to incorporate the enhancing edge of the tumor (12).

A similar voxel was used to acquire an MRS spectral pattern from the opposite normal side of the brain for comparison. The MRS acquisition values used were TE 144 msec and TR 1500 msec.

Reporting of all images was carried out initially by the same MRI consultant radiologist in consensus with the resident radiologist. Subsequently a second MRI consultant radiologist (with similar experience as the first) reviewed all the images independently. The second consultant was blinded to the previous report. Consensus between the two sets of doctors was then arrived at. Both reporting radiologists were blinded to the final diagnosis.

They broadly classified the lesions on MRI first, then MRS combined with MRI. For MRS, this study only used absolute values of choline, creatinin, lactate, lipids and N- acetyl choline plus two ratios; that is, choline: creatinin and N- acetyl choline: creatinin. MRS was not used solely to make any diagnosis.

Lesion classification was broad and not specific. It was based on four categories; one was infection (non-neoplastic), two was degenerative (non- neoplastic), three was neoplastic and the fourth category was indeterminate.

Previous studies have focused on the usefulness of MRS in the characterisation of brain tumours (1) and intra- axial brain masses (8,9). They have also studied the role of MRS in grading tumors for example MRI and choline/creatine ratio discrimination of high and low grade cerebral gliomas (6). MRS biomarkers in the prediction of brain tumour clinical grades have been looked at (7). The MRS spectral pattern of different disease entities has also been reviewed such as in, intracranial infections (22), tumefactive demyelinating lesions (23), stroke (24) and, Acquired Immune Deficiency Syndrome (27), amongst others.

This study's main focus was on the differentiation of neoplastic from non- neoplastic (infections and degenerative conditions) intracranial lesions. A line of thought more practical in our set up because it is a common clinical question, which is aimed at avoiding unnecessary biopsies of non- neoplastic lesions that are indeterminate on MRI.

The gold standard for final diagnosis is histopathology. But, this was not always feasible

because some paediatric tumours are located in the posterior fossa which represents unfavorable anatomical site for biopsy (7); some adult tumours were located in eloquent areas of the brain, for which to insist on a biopsy would expose a patient to high risk. And, infective and degenerative lesions are not commonly biopsied. Therefore, when there was no histological diagnosis, a combination of clinical, laboratory and/or radiological features were used. This combination had to have satisfied the criteria of a final diagnosis.

This criteria was based on improvement or resolution of the condition on treatment (infectious lesions); or imaging follow-up (degenerative lesions). The follow-up was for a minimum of six months.

*Sample size:* Estimated sample size of 70, was calculated using the formula used for minimum desired sample size adapted from the paper by Russell H. Morgan(19). The equation used applies to studies designed to measure the accuracy of diagnostic tests. An assumed accuracy of 90% as adapted from a previous paper that had calculated the percentage agreement between MRI, MRS and final diagnosis in the assessment of brain tumours (26). In order to determine the percentages at which MRI and MRI, MRS agree with the final diagnosis at Aga Khan University, the sample size was determined with 95% confidence(20).

*Analysis*

*Measure of agreement:* Kappa (k) measure of agreement was used to analyse the levels of agreement between MRI and the final agreement, as well as MRI, MRS and the final agreement.

For the two tests, sensitivity, specificity and predictive values with corresponding 95% confidence intervals were calculated on the basis of 2X2 tables. For this calculation, the indeterminate lesions were excluded. The lesions with a diagnosis were grouped into neoplastic and non-neoplastic (infective and degenerative).

The indeterminate lesions on MRI and MRI, MRS, were tabled and the difference in number noted.

Logistic regression demonstrated the role of confounding factors in the diagnostic use of MRS. These confounding factors are morphological characteristics of the brain lesion or surrounding brain that may increase or reduce the yield of MRS.

Specific cases where MRS augmented the diagnostic yield of MRI that resulted in a MRI, MRS diagnosis which corresponded to the final diagnosis are demonstrated.

**RESULTS**

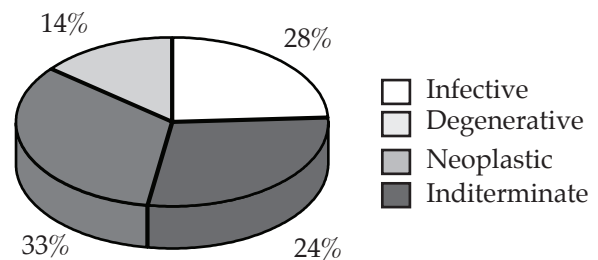
*Study group:* All neoplastic lesions had a histological diagnosis (diagnostic or excision biopsy). Patients

with infective or degenerative cause had a final diagnosis based on resolution of symptoms and signs, laboratory findings and imaging (MRI) follow-up that showed resolution. This assessment lasted not less than six months.

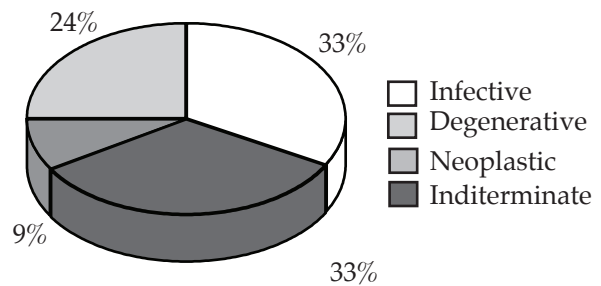
**Figure 1a-c**

*Pie charts. MRI, MRI, MRS and final diagnosis characterisation of intracranial lesions into the four categories (infective, degenerative, neoplastic and indeterminate)*

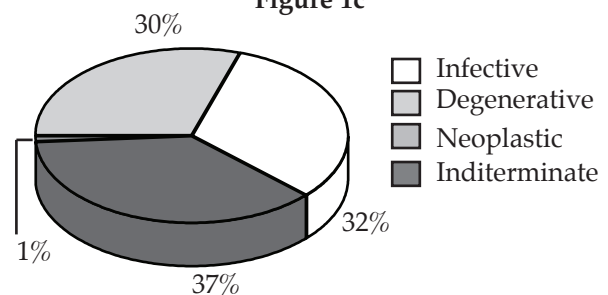
**Figure 1a**



**Figure 1b**



**Figure 1c**



*Kappa:* The measure of agreement between MRI and final diagnosis and that between MRI, MRS and the final diagnosis was 78.9 and 86.3% respectively. *Sensitivity, Specificity, Predictive Values and Accuracy:* Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of MRI were 94.7, 100, 100, 97.9 and 96.1% respectively. Sensitivity, specificity, positive predictive value, negative predictive values and accuracy of MRI, MRS were 99.52, 100, 100, 97.9 and 98.1% respectively. Comparison of the number of lesions categorised as indeterminate lesions by MRI and MRI, MRS.

**Table 1**  
*MRI and MRI, MRS classification of lesions into determinate and indeterminate*

MRI	No of patients (n)	Percentage	MRI,MRS	No of patients (n)	Percentage
Determinate	65	87.8	Determinate	69	93.2
Indeterminate	9	12.2	Indeterminate	5	6.8
Total	74	100	Total	74	100.00

**Table 2**  
*Logistic regression analysis of confounding factors that may affect MRS yield.*

Confounding factors	Odds Ratio	Std. Err	95%confidence interval	P-value
Near fat				
No	Ref		-	-
Yes	4.40	3.60	[.90, 21.39]	0.07
Near csf				
No	Ref		-	-
Yes	2.88	1.90	[.79, 10.51]	0.11
Near bone				
No	Ref		-	-
Yes	1.12	0.77	[-.29, 2.14]	0.38
Solid				
No	Ref		-	-
Yes	0.54	0.38	[1.4, 2.14]	0.38
Enhancing				
No	Ref		-	-
Yes	4.39	3.16	[1.07,17.96]	0.04

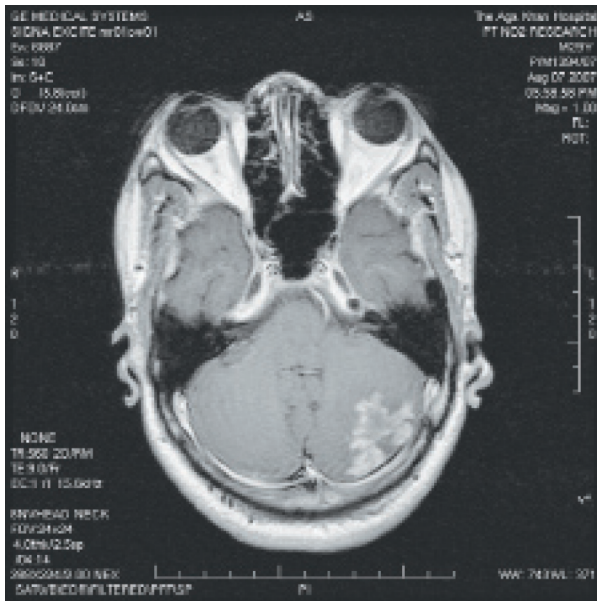
All images depicted are only a selected representation of the whole multisequential, mutiplanar MR study and were in no way used singly to make a diagnosis.

Specific case where MRS augmented the diagnostic yield of MRI (after inter- radiologist

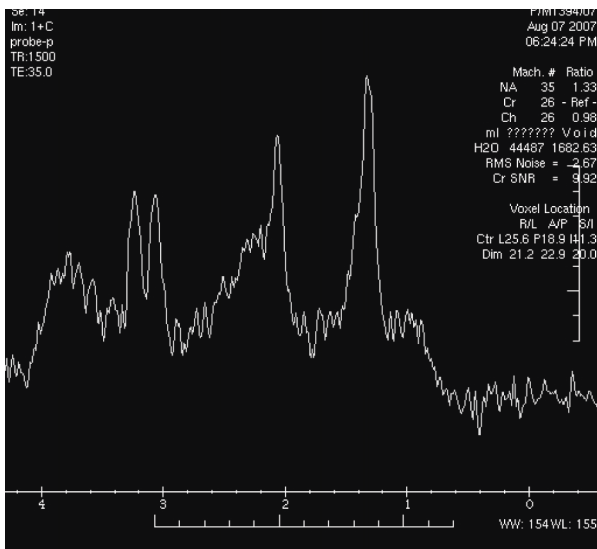
variability), that resulted in a MRI-MRS diagnosis which corresponded to the final diagnosis.

Thirty three year old male with the initials PT, who had headache. MRI conclusion was that the heterogeneously enhancing left cerebella lesion was either a neoplasm or infection.

**Figure 2**  
*Selected axial T1 post- contrast MRI image*



**Figure 3**  
*The corresponding MRS spectrum which when combined with the MRI differential possibilities, raised a higher possibility of an infective cause due to the lactate peak, low NAA:Cr ratio and absence of a choline peak*

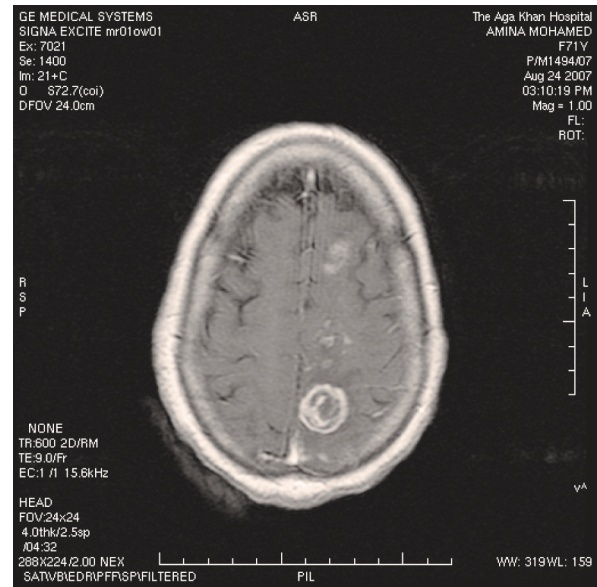


MRI,MRS reported infective cause: Biopsy findings agreed with MRI,MRS by labeling the lesion a tuberculoma. En-quote “Encapsulated caseous mass surrounded by a broad band containing tubercles and Langhan’s giant cells. (Category 1).Illustration of the one of the unusual degenerative (category 2) lesions. 71 year old hypertensive female (AM) who had clinical features suggestive of a cerebral-vascular accident.

MRI showed an area in the left parietal lobe with intense enhancement. This was suggestive

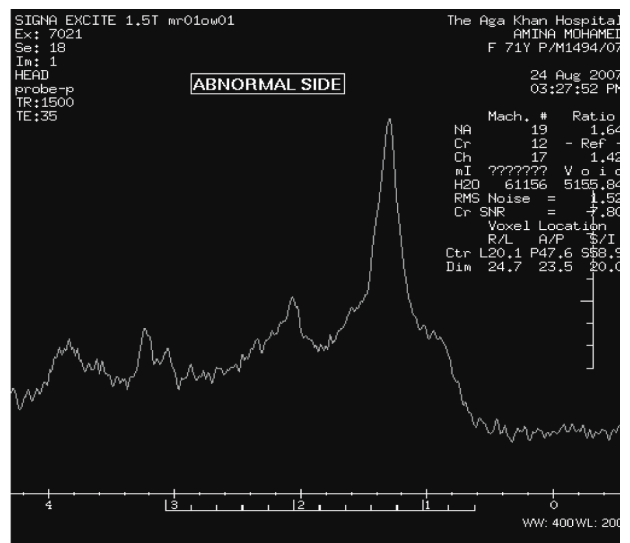
of infection. However, a differential diagnosis of a venous infarct with luxury perfusion was raised. On MRI venography superior sagittal sinus thrombosis was demonstrated.

**Figure 4**  
*Selected axial post contrast T1 image of AM*



MRS spectrum revealed a solitary finding of elevated lactate which when factored into the two possibilities raised by the MRI differential diagnoses,was in keeping with category 2 and not.

**Figure 5**  
*Corresponding MRS spectrum of AM*



Six month follow-up showed that patient improved on conservative treatment for venous infarction without treatment for infection.

## DISCUSSION

MRS is an advanced sequence used in MRI in addition to conventional sequences. Incorporation of this sequence involves additional software in the MR scanner and additional scanning time. For this to improve the diagnostic potential of MR, it seems like a small price to pay. The diagnostic utility of MRS is varied and a lot of aspects are still being researched. This paper sought to investigate the diagnostic utility of MRS when it is used in conjunction with conventional MRI in the differentiation of neoplastic from non-neoplastic lesions, mainly as a way to avoid unnecessary or impractical biopsies. MRS was not used alone to make a diagnosis.

In the comparison of agreement between MRI with the final diagnosis, and MRI, MRS with the final diagnosis, the respective kappa values were 78.9 and 86.3. This showed that there was excellent agreement between the two tests. However there was a 7.4% increase in agreement when MRS was combined with MRI. The corresponding confidence intervals of the two tests did not overlap, therefore, it can be inferred that the increase in agreement that MRS conferred to MRI was of diagnostic value.

In this study, the sensitivities of MRI and MRI, MRS were 94.7 and 99.52% respectively. MRS increased the sensitivity by 4.82%. These values are comparable to those quoted by Schumacher *et al*, in the classification of brain tumour versus non-tumour disease using MRI (94%) (22), and Galanaud *et al*, in a paper which showed a sensitivity of 97%, in the MRI, MRS diagnostic assessment of brain tumours (23). Specificity, positive and negative predictive values of the two tests in this study were similar (100, 94.7 and 97.9%). As compared to the figures published by Schumacher *et al* (22), which had a specificity of 43%, positive predictive value of 96%, and a negative predictive value of 45% and that by Galanaud *et al* which had a specificity of 67% (23), the specificity and negative predictive values of this study were higher. This may have been because of the difference in brain lesion classification.

Accuracies of MRI and MRI, MRS recorded in this study were quite high, at 96.4 and 98.1% respectively. A 1.7% increase in accuracy was conferred by MRS. An accuracy quoted by Galanaud *et al* (23) of 90% is lower. This may also be due to the difference in lesion classification as stated above.

The potential benefit of MRS in the reduction of indeterminate lesions is demonstrated in the pie charts illustrating the four categories as classified by the two tests. Further analysis showed that the addition of MRS to MRI reduced the number of indeterminate lesions by 5.4%. This shows that MRI, MRS can reduce the number of cases in need of a biopsy when there is doubt on MRI, as to whether a lesion is neoplastic or non-neoplastic. This role of

MRS is redemonstrated in a study published by Lin *et al*, that showed that MRS avoided stereotactic biopsy in some patients with suspected or already treated brain tumours (24).

When the kappa agreement, sensitivity, specificity, predictive values and accuracy, as well as the reduction in indeterminate lesions are combined, we can infer that MRS has diagnostic utility in the improvement of the categorisation of focal brain lesions into neoplastic and non-neoplastic.

As reiterated by Hou *et al* (25), MRS complements conventional MRI in the characterisation of brain pathology. The cost effectiveness of additional ten minutes to an MRI head examination resulting in higher diagnostic yield is worthwhile.

While various comparisons have been made to this study, it is important to note that there were distinct differences in the objectives and characterisation of brain lesions. Majority of the previous studies looking at the role of MRS in intracranial pathology have had various objectives that can be broadly classified in three categories.

First, description of MRS spectra of specific disease entities. Secondly, the clinical use of MRI, MRS without comparison to MRI alone. For example, 'MRI, MRS of Human Brain Tumours' (25) and; 'Discrimination between neoplastic and non-neoplastic brain lesions by use of proton MR spectroscopy: the limits of accuracy with a logistic regression model' (26). Similarly, in a study published by Sibtain *et al* on the clinical value of MRS in adult brain tumours, the literature is reviewed regarding the role of MRS in the diagnosis of brain tumours (27), third, the clinical use of a combination of advanced MR imaging techniques, including MRS.

Not much literature has been tailored to the specific objective of this study. This has limited the level of direct comparison.

Logistic regression for analysis of lesion location and morphological factors that may affect MRS yield showed that only the presence of enhancement has a p-value of less than 0.05, hence it is the only parameter that was found to be significant in MRS voxel position placement. This is reiterated in a study done on the effect of voxel position on single-voxel MRS findings by Ricci *et al* (17). Close proximity of the lesion to fat had an odds ratio of 4.4 but the p-value was 0.7. Because the standard error is high, we can infer that if the sample size had been larger, this may also have been a significant characteristic. However, literature did not reveal any studies to this effect. The other morphological characteristics were shown to be statistically insignificant.

In the classification of lesions on MRI, inter-radiologist variability only happened in two patients. The radiologists came to a consensus after including the MRS findings. There was no variability in the MRS interpretation or MRI, MRS diagnoses.

There were some limitations in this study. First, it is recognised that though the preferred final diagnosis was histology, this was mainly achieved in the neoplastic lesions. And, these were not subjected to pathologist inter-observer variability. Second, MRS interpretation was limited to the metabolites available for interpretation. These were dependent on the MRS software available to the department. The metabolites available for interpretation were not all that are currently used for optimal MRS analysis. However, as stated by Butzen *et al*, the most accurate method of clinical MRS interpretation remains an open question (26). Third, in the acquisition of the MRS spectra, there was no quality control calibration of the MRS studies using a phantom prior to the MRS scans. This may have contributed to the irregular MRS spectral baselines, which is usually due to inadequate suppression of water molecules.

In conclusion, additional use of MRS improves the diagnostic value of MRI in the differentiation of neoplastic from non- neoplastic (infective and degenerative) focal brain lesions. MRS was useful to arrive at a more definitive diagnosis in these lesions with doubtful morphological imaging patterns.

#### ACKNOWLEDGEMENTS

To the patients who patiently underwent the examinations for this exercise and all the MRI staff for the role they played. The authors were supported by the Aga Khan University Hospital, Nairobi Research and Ethics Committee.

#### REFERENCES

- Hollingworth, W., Medina, L. S., Lenkinski, R. E., *et al*. A Systematic Literature Review of Magnetic Resonance Spectroscopy for the Characterisation of Brain Tumours. *Amer. Soc. Neuroradiol.* 2006; **27**: 1404-1411.
- Warren, K. NMR Spectroscopy and Pediatric Brain Tumors. *The Oncologist* 2004; **9**: 312-318.
- Farr RFA-RP. Physics for medical Imaging, 2nd edition. *Institute of physics publishing*; 2007.
- Cha, S. Update on Brain Tumor Imaging: From Anatomy to Physiology. *Amer. J. Neuroradiol.* 2006; **27**: 475-487.
- IR, Y. Review of modalities with a potential role in radiology. *Radiology* 1994; **192**: 307- 317.
- Fayed, N., Morales, H., Modrego, P. J. and Pina, M. A. Contrast/Noise ratio on conventional MRI and choline/creatine ratio on proton MRI spectroscopy accurately discriminate low-grade from high-grade cerebral gliomas. *Acad. radiol.* 2006; **13**: 728-737.
- Astrakas, L. G., Zurakowski, D., Tzika, A. A., *et al*. Noninvasive Magnetic Resonance Spectroscopic Imaging Biomarkers to Predict the Clinical Grade of Pediatric Brain Tumors. *Clinical Cancer Research*; 2004; **10**: 8220-8228.
- Ali-Okaili, R. N., Kregja, J., Woo, J. H., *et al*. Intraaxial brain masses. MR imaging- Based diagnostic study- Initial experience. *Radiology.* 2007: 539-550.
- Al-Okaili, R. N., Kregja, J. and Wang, S. Advanced MR imaging techniques in the diagnosis of intracranial tumours in adults. . *Radio Graphics.* 2006: 539-550.
- Palumbo, B., Lupattelli, M., Pelliccioli, G. P., *et al*. Association of 99mTc-MIBI brain SPECT and proton magnetic resonance spectroscopy (1H-MRS) to assess glioma recurrence after radiotherapy.
- The quarterly journal of nuclear medicine and molecular imaging: official publication of the Italian Association of Nuclear Medicine (AIMN) [and] the International Association of Radiopharmacology (IAR), and Section of the Society of Radiopharmaceutical Chemistry and Biology. **50**: 88-93.
- Graves, E. E., Nelson, S. J., Vigneron, D. B., *et al*. Serial Proton MR Spectroscopic Imaging of Recurrent Malignant Gliomas after Gamma Knife Radiosurgery. *Amer. J. Neuroradiol.* 2001; **22**: 613-24.
- Delorme, S., MA. W. Applications of MRS in the evaluation of focal malignant brain lesions. Department of Radiology (E010), Heidelberg, Germany. 2006; **22**: 95-99.
- SJ, N. Multivoxel Magnetic Resonance Spectroscopy of brain tumors. *Molecular Cancer therapeutics.* 2003; **2**: 497-507.
- MA P. Advanced kidney disease, gadolinium and nephrogenic systemic fibrosis: the perfect storm. *Current Opinions in Nephrology and Hypertension.* 2009.
- Prince, M. R., Zhang, H., Morris, M., *et al*. Incidence of nephrogenic systemic fibrosis at two large medical centers. *Radiology.* 2009; **2**: 612-613.
- Colletti, P. M. Magnetic Resonance Procedures and Pregnancy. [Reviw updated 2001; cited].
- Ricci, P. E., Pitt, A., Keller, P. J., *et al*. Effect of Voxel Position on Single-Voxel MR Spectroscopy Findings. *Amer. J. Neuroradiol.* 2000; **21**: 367-374.
- Panigraphy, A., Krieger, M.D., Gonzalez-Gomez, I.U., *et al*. Quantitative Short Echo Time 1H-MR Spectroscopy of Untreated Pediatric Brain Tumors: Preoperative Diagnosis and Characterization. *Amer. J. Neuroradiol.* 2006; **27**: 560-572.
- Eng, J. Statistical concepts series. Sample size estimation. How many individuals may be studied? *Radiology.* 2003; **227**: 309-313.
- Mugenda, O. and Mugenda, A. Research Methods. Quantitative and Qualitative Approaches. Nairobi: *African Centre for Technology Studies*; 2003.
- Shellock, F. G., Parker, J. R., Pirovano, G., *et al*. Safety characteristics of gadobenate dimeglumine: clinical experience from intra- and interindividual comparison studies with gadopentetate dimeglumine. *J. Magn. Reson. Imaging.* 2006; **6**: 1378-1385.
- Schumacher, M. Magnetic Resonance Imaging compared with biopsy in the diagnosis of brainstem diseases of childhood. *J. Neurosurgery.* 2007; **106**: 111-119.
- Galanaud, D., Nicoli, F. and Chinot, O. Non invasive diagnostic assessment of brain tumours using combined in vivo MRI and MRS. *Magnetic Resonance in Medicine.* 2006; **55**: 1236-1245.

25. Lin, A., Ross, B. D. and Harris, K. Efficacy of Proton Magnetic Resonance Spectroscopy in Neurological Diagnosis and Neurotherapeutic Decision Making. *NeuroRx*. 2005; **2**: 197-214.
26. Hou, B. and Hu, J. MRI and MRS of Human Brain Tumors. *Methods in molecular biology*. 2009; **520**: 297-314.
27. Butzen, J., Prost, R. and Chetty, V. Discrimination between neoplastic and nonneoplastic brain lesions by use of proton MR spectroscopy: the limits of accuracy with a logistic regression model. *Amer. J. Neuroradiol*. 2000; **21**: 1213-1219.