MAGNETIC RESONANCE SPECTROSCOPY (MRS) BASED TARGET VOLUME DELINEATION OF HIGH GRADE GLIOMAS

DEPARTMENT OF RADIATION THERAPY

CHRISTIAN MEDICAL COLLEGE, VELLORE

CERTIFICATE

This is to certify that **"Magnetic Resonance Spectroscopy based target volume delineation of High Grade Gliomas"** is an original work by **Dr.Uday Krishna.A.S** in partial fulfilment towards M.D. Radiotherapy (Branch IX) Degree examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2012.

GUIDE:

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Prof. Dr Subhashini John, DMRT, MD,MNAMS

Professor & Head of Department

Department of Radiation Oncology

Christian Medical College, Vellore

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AIMS AND OBJECTIVES OF THE STUDY

1) The primary objective is to assess the changes in the radiation target volume by addition of Magnetic Resonance Spectroscopy when compared to Computerised Tomography (CT)-Magnetic Resonance Imaging (MRI) alone in treatment planning of High Grade Gliomas (HGG).

2) The secondary objective of this study is to examine the feasibility of incorporating Magnetic Resonance Spectroscopy imaging into the routine treatment planning process.

INTRODUCTION TO THE STUDY

Malignant gliomas are the most common primary brain tumour in adults. Survival of patients with high grade gliomas has been dismal inspite of advancements in treatment techniques. Optimal treatment of this subset of gliomas involves maximal safe resection followed by post operative therapy in the form of radiation and chemotherapy.

Walker et.al in the 1970s showed a survival benefit of 6 months by addition of post operative whole-brain radiotherapy (WBRT) after maximal safe resection of high grade gliomas(HGG). Prognosis of HGG was dismal with median survival of about 9 to 12 months for grade IV tumors[1]. Local control has been very poor with radiation therapy only [2]. Invention of CT and MRI led to change in concept of post op radiotherapy volume and this shifted from whole brain radiotherapy to image guided focal radiation to the post op bed rather than whole brain radiotherapy.

A recent EORTC-NCIC study on HGG by Stupp et al showed that with adjuvant radiation and concurrent and adjuvant temozolomide survival of HGG improved to 27% at 2 years and update in 2009 gave results at 5 years follow up which was around 15%[3]

Autopsy studies conducted on patients of high grade glioma showed that majority of patients had tumor recurrences around the resection cavity and that inclusion of whole brain in the radiation portal led to radiation induced damage to the normal brain substance. In view of the above features there was a shift in trend towards using higher imaging modalities for localisation of primary tumor and more conformal radiation techniques.

There are uncertainities concerning the optimal target volume in Glioblastoma multiforme.The safety zone around the preoperative contrast enhancing tumor vary quite significantly between different institutions ranging between 1-4 cm upto the complete peritumoral edema and 2-3 cm safety margin. This was shown in terms of the studies that showed that maximum tumor recurrences occurred about 2-3 cm around the resection cavity.

To utilise the benefits of high precision radiation techniques, it is critical to define the regions of active tumor (GTV-Gross Tumor Volume) that needs a higher dose, areas of suspicious tumor extension (CTV-Clinical Target Volume) that needs an intermediate dose and critical structures that need lower dose (OAR-Organs at Risk). The uniform margin of 2-3 cm around the contrast enhancing high grade lesion on computerised tomography scan (CT) has been the current practice in high grade gliomas that may lead to underdose of CTV at places with increased dose to normal brain tissue.

With advent of Magnetic Resonance Imaging (MRI) this uniform margin was changed to 2-3 cm around the enhancement in T2W FLuid Attenuated Inversion Recovery (FLAIR) sequences followed by additional boost to the enhancing component on post gadolinium series. Disadvantages posed by MRI imaging was that the gadolinium enhancement just implies the breakdown of blood brain barrier and with T2W images either overestimated or underestimated the microscopic spread along the white matter fibre tracts leading to inclusion of large volume of normal brain in the radiation portal. This led to a need for molecular imaging for better target delineation.

Magnetic resonance spectroscopic (MRS) imaging is an emerging powerful tool for target definition in brain tumors. It provides regional information about the tumor pathology based on levels of cellular metabolites that includes choline, creatine, NAA, lipid and lactate. Gliomas exhibit a high resonance in the spectral region of choline and low NAA and creatine resonance implying increases in choline: creatine and choline: NAA ratios. In the context of radiotherapy, MRS has several potential applications. These include, identifying tumour extent and metabolically active regions that includes distinguishing tumour from normal tissue, benign lesions, for evaluating response to treatment , for identifying recurrence and radiation necrosis.

A pilot study was done to assess the changes in the target volumes of High Grade Gliomas with addition of MRS to CT-MRI based planning when compared to CT-MRI alone**.**

3. Review of Literature

3A. Demography of CNS Tumors in the World and in India

Two major data sources can be considered to define the statistics of brain tumors in the world. They include the Surveillance, Epidemiology and End results (SEER) program of the National Cancer Institute and the Central Brain Tumor Registry of United States (CBTRUS).

The SEER registry reports that the incidence of primary CNS tumors is between 2.2 and 8.3 per 100,000 people per year[4]

The CBTRUS incidence of new CNS tumors in the United States is 43,800 cases per year that includes both benign and malignant histologies in their assessment [5]

Brain cancer constitutes about 3.5% of all cancers in Indian subcontinent (4.34% in males and 3% in females)[6 7] High grade gliomas (HGG) constitute about 46 % of all the histological types[8] It is seen in the adult population age 35 to 64 years with an increasing percentage with advancing age[9]

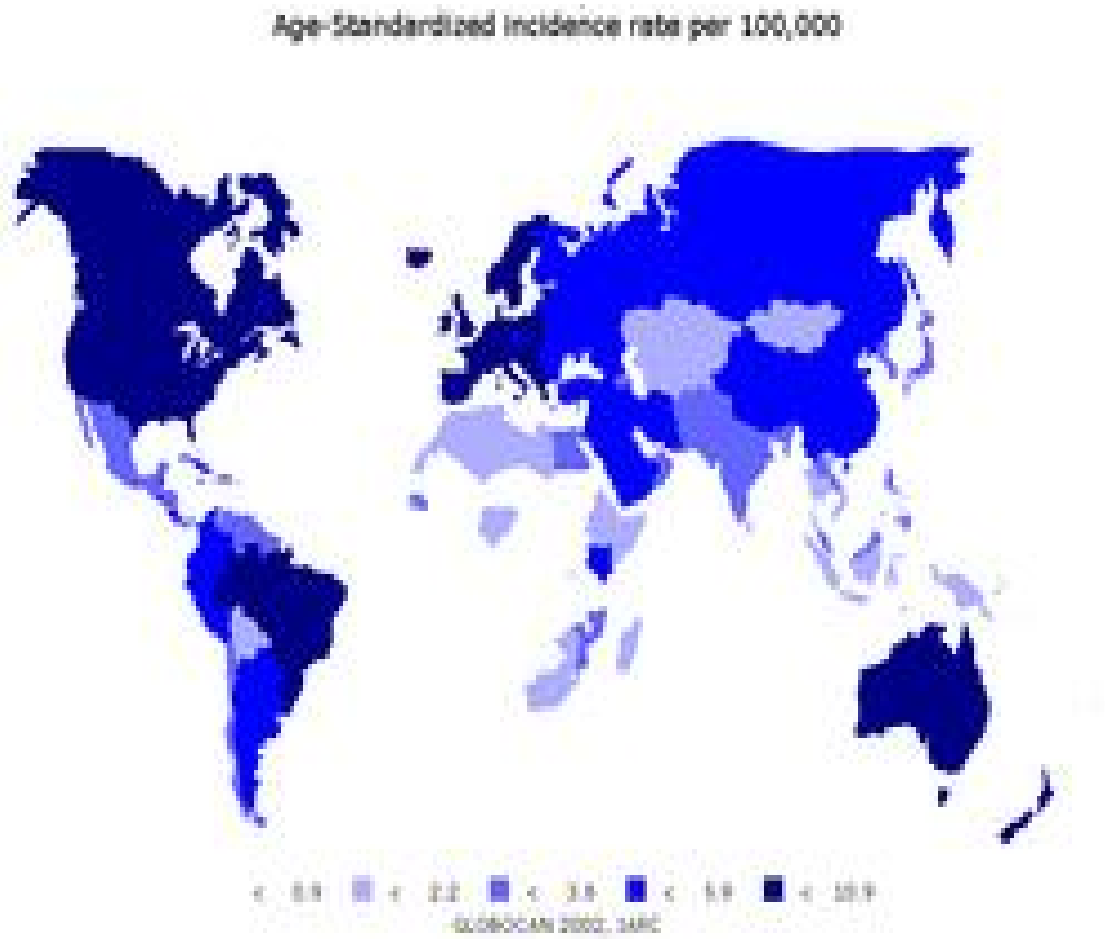
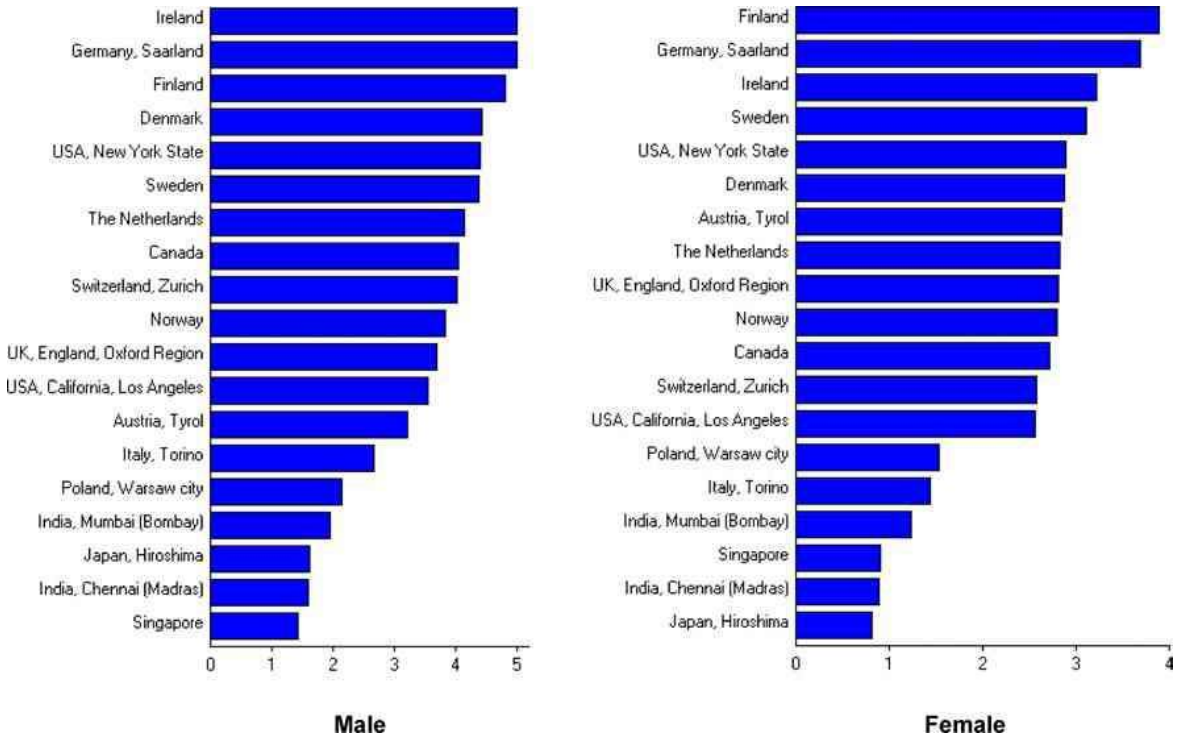


Figure 1: Age standardised incidence per 1,00,000

Figure 2: Worldwide incidence of High Grade Gliomas



3B. <u>Imaging Characteristics of High Grade Gliomas</u>

1. Computerised Tomography (CT)scan is used in post op setting to assess for haemorrhage , or residual disease.It is the imaging of choice for radiotherapy treatment planning .It is utilised as primary imaging modality in those patients with inability to undergo MRI (implanted pacemaker, metal fragment, paramagnetic surgical clips) or unwilling because of claustrophobia to undergo MRI.

2. MRI is the most powerful tool in neuro imaging. It involves using a radiofrequency pulse to escite hydrogen protons within a magnetic field to elevate the protons to a higher energy state. On returning to the resting lower energy state they release electromagnetic energy.This is recievd by a coil or antenna and converted into image.The time to return fromn excited state is called relaxation time (TR).

Standard sequences on MRI include –

T1W

T2W fast spin echo

T2W FLAIR and

DWI

T1W- demonstates normal anatomy and is performed with contrast gadolinium. This accumulates where there is breakdown of Blood Brain Barrier.

T2W – Fast spin echo sequences highlight tissues with a high concentration of water as a bright signal or hyperintensity. In FLAIR, CSF is suppressed and lesion conspicuity is improved.

GBM shows vasogenic edema and ring enhancement around central necrotic regions. Anaplastic gliomas show ring enhnacement like GBM or no enhancemenmt like low grade gliomas.

DWI -measures the mobility of water molecules. Water mobility isdescribed using a scalar parameter, the diffusioncoefficient D. Apparentdiffusion coefficient (ADC) can becomputed from a pair of images with orwithout additional diffusion sensitizationgradient pulses.

3. Applications of MRI include MR angiography and venography, Magnetic resonance spectroscopy (MRS), Functional MRI (fMRI), and MR perfusion.

4. PET involves adminitration of cyclotron produced F-18 labelled FDG which competes with serum glucose for cellular uptake in the body. With FDG uptake in the cells of the tumor, it becomes phosphorylated by the enzyme hexokinase which slows down the degradation of FDG by the glycolytic cycle and reduces the diffusibility of FDG out of the cell. PET imaging using amino acids such as tyrosine or methionine as well as choline tagged with Carbon 11 are experimental and clinical efficacy is yet to be proven.

Imaging of brain falls into five different categories which are in initial presentation, prior to surgery, immediate post op period, before initiating chemoradiation, post adjuvant therapy for assessment of disease status and in all these categories, MRI has been found to be superior to CT scan.

Burger et al showed that infiltrating margins of the tumor often extend into regions of brain appearing normal on CT scan. On the other hand an abnormality on a CT did not contain tumor always. Potential pathways of spread of GBM are through white matter tracts, subependymal extension with CSF contamination or haematogenous dissemination[10]

Lee et al showed that when compared to CT scan, MRI is a better imaging modality to delineate the local gross extent of primary brain tumors. MRI signal abnormality was larger than CT abnormality in 62% of patients and 10 patients with equivocal CT scan had clear abnormalities on MRI[11]

Figure3: Post gadolinium MRI of glioma showing rim enhancement

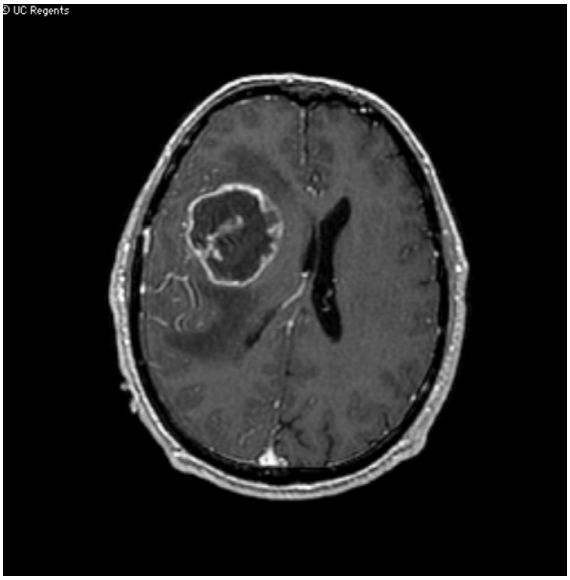
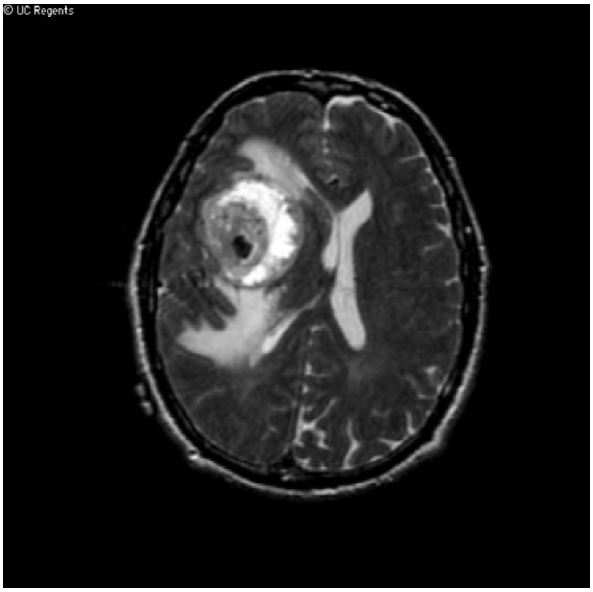


Figure 4: T2W FLAIR showing hyperintensity in the edema around the lesion



3C. <u>WHO Classification of Gliomas</u>[12]

WHO classification of CNS tumors includes a grading scheme that is a malignancy scale ranging a wide variety of neoplasms. The first classification was edited by Zulch in 1979. The second clkassification introduced the advances of immunohistochemistry into diagnostic pathology and was edited by Kleihues et al. The third edition edited by Kleihues and Cavenee in 2000 incorporated genetic profiles into pathology. The current fourth edition of classification was published in 2007 by David Louis et al.

Grade I - low proliferative potential and the possibility of cure following surgical resection.

Grade II – infiltrative in nature and often recur despite complete surgical resection. Some of these can transform to higher grades of malignancy.

Grade III - Nuclear atypia and brisk mitotic activity is the hallmark and these lesions need adjuvant radiation +/- chemotherapy.

Grade IV – cytologically malignant, mitotically active, necrosis prone neoplasms typically associated with pre and post op disease evolution and fatal outcome.

The International Classification of Diseases of Oncology (ICD-O) codes for various high grade gliomas are as follows:

Anaplastic Astrocytoma – 9401/3

Anaplastic Oligodendroglioma – 9451/3

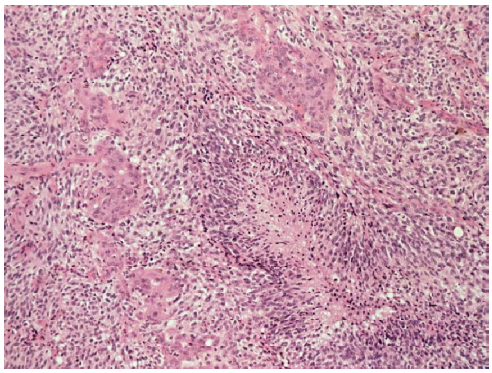
Anaplastic Oligoastrocytoma – 9382/3

Glioblastoma – 9442/3

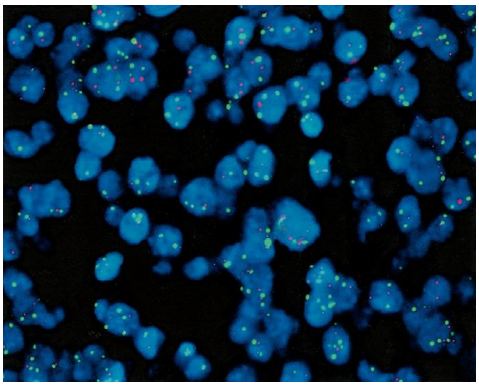
(3 implies the malignant behaviour of the tumor)

3D. <u>Pathological Characteristics of High Grade Gliomas</u>

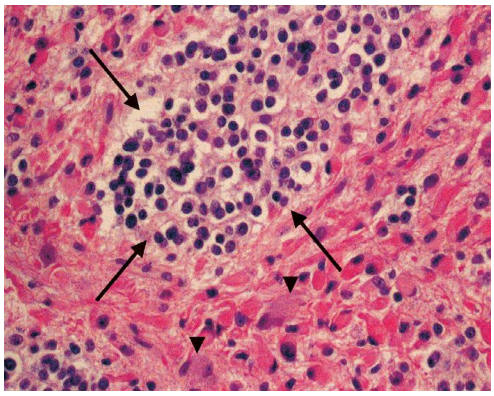
<u>Anaplastic</u> Oligodendrogliomas are composed of isomorphic cells with round, hyperchromatic nuclei.They have clear cells with central spherical nuclei and well defined cell borders, the so-called honeycombappearance.They demonstrate a typical vascularisation pattern consisting of dense network of branching capillaries resembling chicken wire pattern .Other features are perineuronal satellitosis, perivascular aggregates. They are positive for S-100, CD57, MAP2, Olig1 andOlig2. They lack nuclear staining of p53 and the MIB labelling index is about 5%.



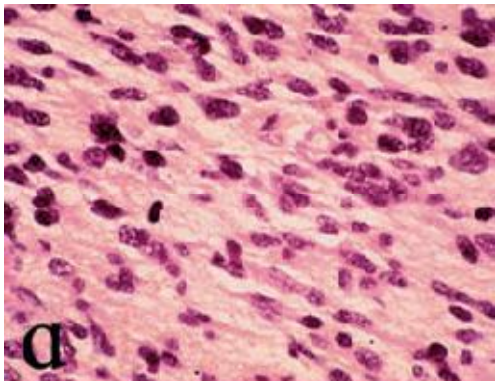
Flouroscent in situ hybridisation showing 1p 19q codeletion status



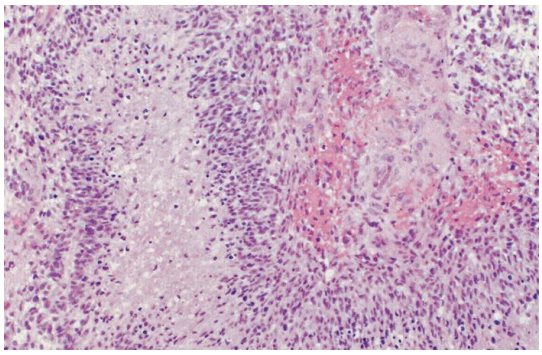
Anaplastic Oligostrocytoma - shows a diffusely infiltrating oligoastrocytoma with focal or diffuse histologicalsigns of anaplasia, such as high cellularity,nuclear atypia, cellular pleomorphism, obvious mitoticactivity and microvascular proliferation .



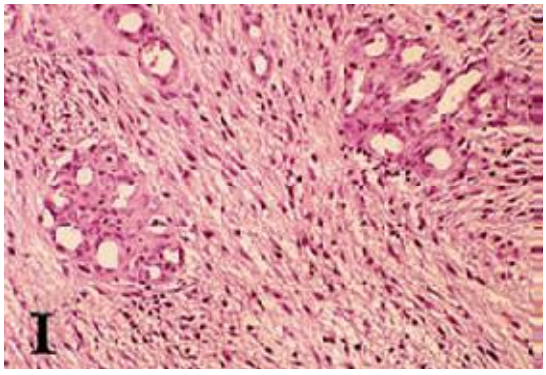
Anaplastic Astrocytoma- Histologically, these tumors have nuclear atypia and mitotic activity, without necrosis or neovascularization. They are GFAP positive and positive for protein S-100. Nuclear p53staining is found in about 60% of the cases. Stainingfor Ki-67 (MIB1) usually shows nuclear positivity inmore than 5% of the tumor cells.



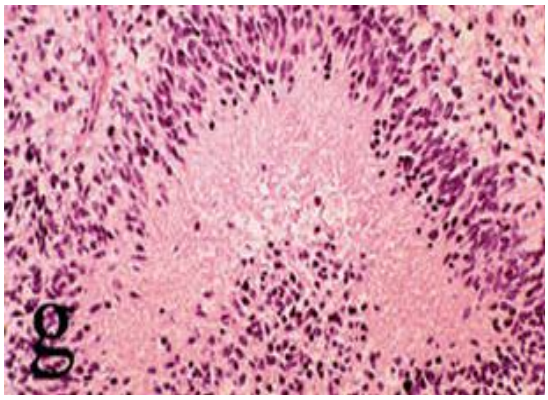
Glioblastoma – gross pathology is characterised by nuclear atypia, mitotic activity, vascular proliferation and necrosis.They are positive for GFAP and S-100.Nuclear reactivity for P53 is seen in 30-40% of all glioblastomas and MIB labelling index is usually high (10%).



Microvascular proliferation with a glomerulum- or garland-like capillary structure



Necrosis with pseudopalisading pattern



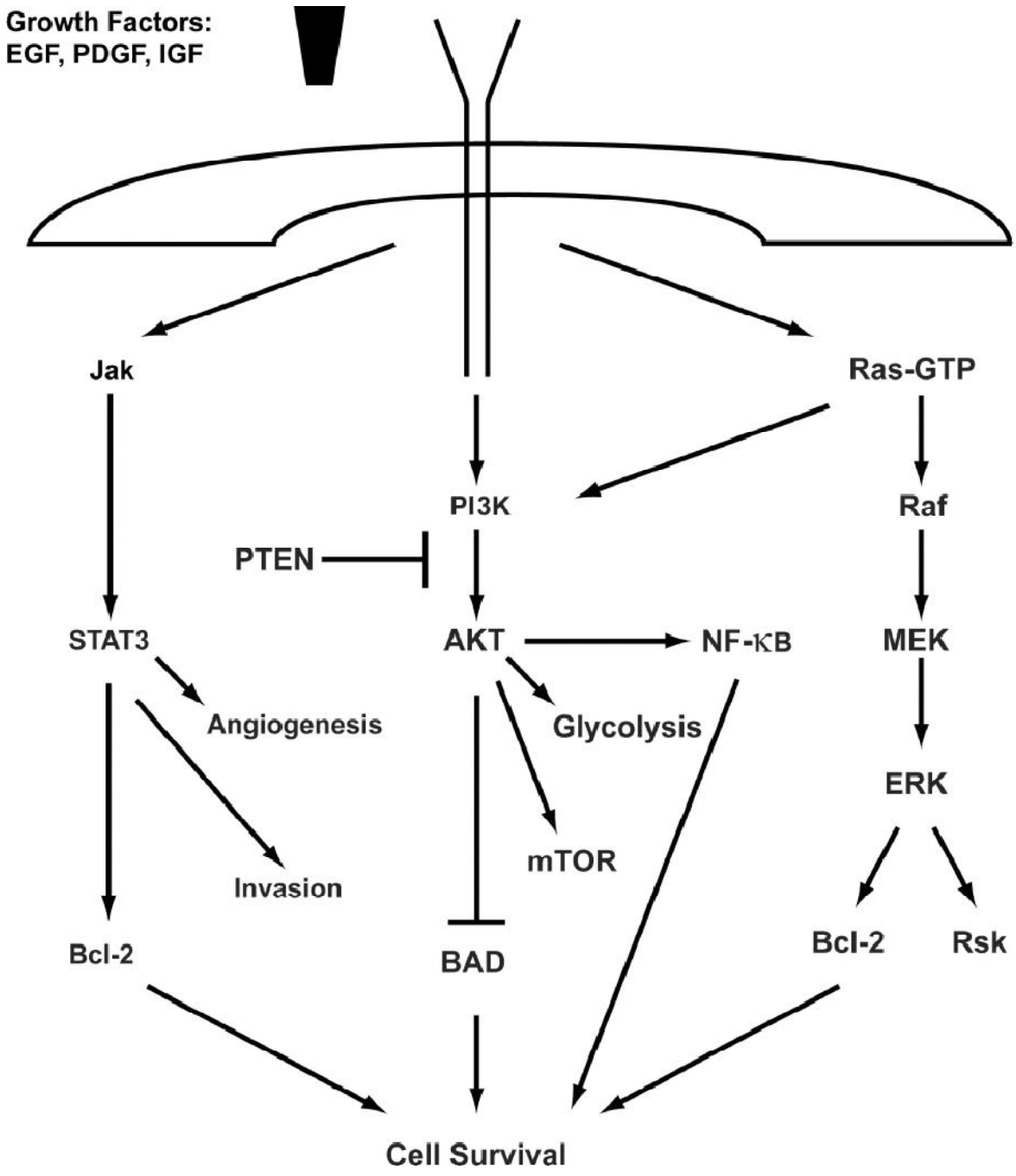
3E.Molecular Biology of Glioma and process of progression to HGG

Gliomagenesis is charecterised by astrocytes undergoing transformation with the loss of tumor suppressor genes critical for cell growth, differentiation, and function. These genes are TP53, the retinoblastoma (RB) gene, the INK4a (inhibitor of cyclin-dependent kinase 4) gene, and the PTEN gene.

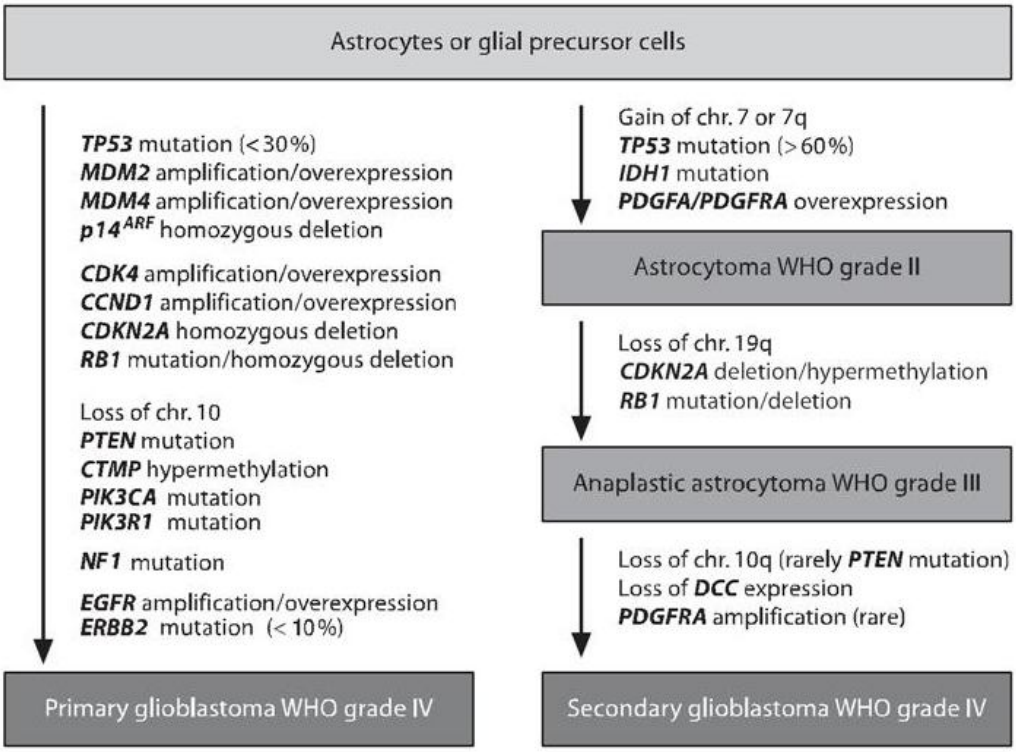
In low-grade astrocytomas TP53 is rendered inactive by gene mutation or gene deletion. This critical step in transformation is found in approximately 60% of low-grade gliomas. The progression into anaplastic astrocytoma and glioblastoma multiforme (GBM) the secondary GBM is by RB and PTEN mutations with cell aneuploidy and overexpression of cyclin-dependent kinase 4 (CDK4).40% of GBM are secondary GBM and the average patient age is younger.

Primary GBM often (60%) show amplification of the epidermal growth factor receptor (EGFR), deletions in the INK4a gene with loss of p14 and p16, and diploid cells. In addition, PTEN mutation is far more common in primary GBM compared with secondary GBM, approximately 25% versus 4%. The loss of heterozygosity in chromosome 10q causes inactivation of PTEN, a gene downstream of focal adhesion kinase (Fak) that controls cell migration and invasiveness. This effect is mediated by activating Akt, a serine/threonine (ser/thr) kinase involved in cell proliferation and survival. The loss of heterozygosity of chromosome 10 has been shown in several studies to have a highly significant and independent impact on prognosis[13,14,15,16,17]

Major pathways that mediate the cell survival in High Grade Gliomas



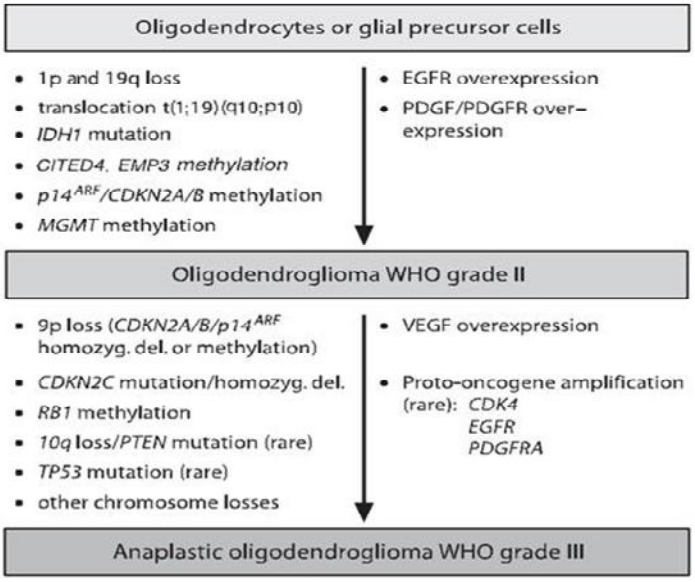
Molecular biology of transformations of astrocytomas is depicted in the figure:



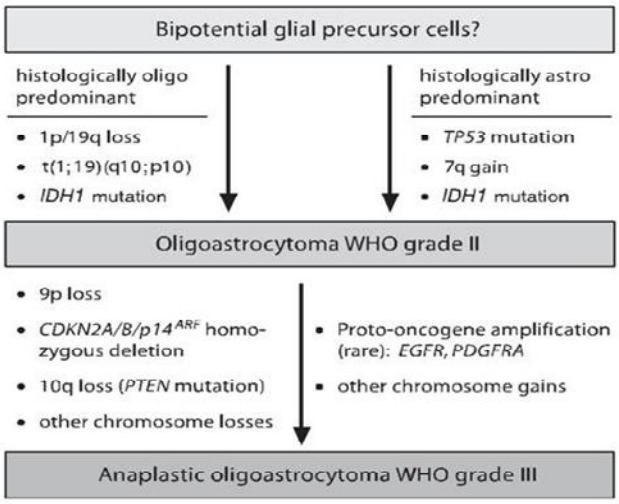
Molecular biology of anaplastic gliomas:

Allelic loss of 1p and 19q is the early genetic alteration in the trasformation of oligodendrogliomas. It is found in 63% in anaplastic ODG, 52% in anaplastic oligoastrocytoma and 8- 11 % for pure astrocytic tumors. It confers a longer PFS, chemosensitivity and radiosensitivity[(18),(19)]

Molecular biology of Oligodendroglioma



Molecular pathology of Oligoastrocytoma



3F .Management of High Grade Gliomas

Role of Surgery in High Grade Gliomas:

HGG are heterogenous and the therapy is guided by the most aggressive grade in the specimen. In view of this obtaining an accurate diagnosis is very essential.

Jackson et al have shown that complete resection of glioma is required for high grade diagnosis and to detect an oligodendroglial component in a tumor that carries prognostic significance[20]

Resection relieves mass effect and an extensive resection is associated with greater neurological improvement.

Randomised trials have shown that cytoreduction with surgical resection provides modest survival benefit. Vourinen et al showed that the estimated median survivial time was 171 days after craniotomy vs 85 days after biopsy alone.The estimated survival time was 2.757 times longer after craniotomy[21] Stummer et al showed that patients without residual contrast enhancing tumor had a higher median overall survival 17.9 vs 12.9 months compared to that with residual tumor[22]

Barker et al have shown that post op radiotherapy is more favourable and that deterioation during treatment is unlikely after resection[23]

Role of Chemotherapy in High Grade Gliomas:

Chemotherapy for Anaplastic Oligodendrogliomas : Cairncross et al through RTOG 9402 have shown that in patients with codeletion of 1p19q , PCV chemotherapy administered prior to radiation showed that progression free survival was better (2.6 years vs 1.7 years) in the arm receiving PCV prior to chemotherapy and this was influnced by codeletion status of 1p19q[24] Van den bent et al studied the benefit of addition of PCV regimen after post op radiation in grade III ODG and found that the progression free survival was 23 mths vs 13.2 mths in the arm receiving both and this was not influenced by codeletion status[25] Vogelbaum et al in RTOG 0131 studied effect of temozolomide in grade III gliomas and found that objective response rate was 33%[26]

Chemotherapy for Glioblastoma: Stupp et al in a randomised trial compared post op radaition alone versus radiation with concurrent and adjuvant temozolomide and showed that 2 year survival was increased by 16% with addition of temozolomide with RT in glioblastoma multiforme[27] These results have changed the standard of care for treatment of glioblastoma multiforme patients. A companion correlative laboratory study demonstrated that methylation of promoter region of methyl guanine methyl transferase (MGMT) gene in the tumor specimen is associated with superior survival regardless of treatment received but benefit was maximal for patients with MGMT promoter region methylation[28]

Targeted therapy in High Grade Gliomas:

Studies on EGFR have shown that patients whose tumors demonstrate the variant 3 mutant (EGFRvIII), with resulting constitutive activation of EGFR tyrosine kinase activity, along with intact phosphatase and tensin analog (PTEN), appear to be somewhat more responsive to treatment with EGFR inhibitors[29]

Angiogenesis inhibitors like Bevacizumab in combination with Irinotecan has shown improvement in PFS rate of 30% at 6 months in patients with GBM[30]

3G.Evolution, techniques and principles of Radiotherapy

Randomised trials have demonstrated clear benefit of post op radiotherapy in high grade gliomas[31]. Autopsy studies and surgical series showed disease recurrence at other sites in the brain and this led to inclusion of whole brain volume in the radiation portal[32]

With advancement in computerised tomography (CT) studies showed that majority of recurrences occurred within 2 cm of the margin of primary tumor[33] Halperin et al did biopsy studies from patients with high grade gliomas from 1 cm and 3 cm beyond the contrast enhancement on the CT scan and found out that 1 cm margin beyond the enhancement covered tumor adequately in 50% of the patients and a margin of 3 cm covered the tumor adequately in 100% of the patient population[34] In view of the above observations, inclusion of tumor and surrounding edema as seen radiologically and margins beyond this has been the radiation volume in high grade gliomas.

Walker et al did a dose response analysis in high grade gliomas and found out that by increasing the total dose from 45 Gy to 60 Gy there was improvement in median survival from 28 weeks to 42 weeks[35] Increase in total dose beyond 60 Gy did not show any improvement in survival[36]

Dose intensification strategies in High Grade Gliomas (HGG):

Role of conformal techniques – 3DCRT and IMRT:

Chan et al studied dose escalation to 90Gy with use of conformal techniques and did not find any advantage in terms of local control although there was significant reduction in the normal tissue toxicity [37] Use of accelerated fractionation strategies along with conformal radiotherapy techniques were tried in RTOG 8302 trial and the results showed that with respect to median survival time there were no differences between the conventional fractionation and altered fractionation [38]

Role of stereotactic radiosurgery (SRS) and stereotactic radiotherapy (SRT) in HGG:

A large study investigated effect of addition of SRS boost to conventional external beam radiotherapy and observed that there was no added advantage in terms of survival, quality of life or decline in cognition [39]

RTOG 0023 tested the toxicity efficacy and feasibility of addition of once weekly FSRT to conventional external beam radiotherapy in patients with GBM and found that it was feasible and well tolerated but there was no survival advantage [40]

Role of Brachytherapy:

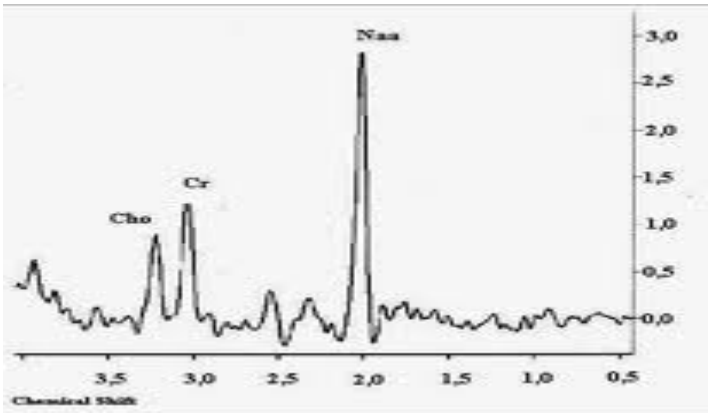
Studies with usage of radioactive iodine for interstitial brachytherapy and Gliasite (inflatable balloon with liquid radioactive iodine) in the post op cavity – as boost to external radiotherapy did not show any survival advantage[41][42]

Role of particle therapy:

RTOG studied the survival benefit by addition of fast neutron boost after conventional external radiotherapy of 50 Gy and found that even though at autopsy there was no tumor in those patients who underwent neutron boost, there was no added survival advantage by addition of neutron boost after external beam radiotherapy [43]

3H. <u>History of Magnetic Resonance Spectroscopy</u>

Magnetic Resonance Spectroscopy uses radiofrequency excitation in presence of magnetic field to obtain signals from naturally occuring proton nuclei (H1) to identify and quantitate metabolites. Most commonly acquired H1 – nuclear magetic resonance spectroscopy of brain have three peaks- the cholines (choline, glycerophosphocholine and phosporyl choline), the creatines (creatine, phosphocreatine) and the NAA (N-acetyl aspartate). Studies of extracts of human astrocytic tumors indicated an increase in the ratio of choline and creatine and reduction in NAA [(44)] A large multicentre study indicated similar correlations in metabolic features in vivo [(45)]



Initial clinical application of MRS was for differentiating recurrent orresidual brain tumor from delayed cerebral necrosis. In this study MRS showed sensitivity for tumor of 71% with a specificity of 100%[(46)]

Usage of Magnetic Resonance spectroscopy in radiotherapeutic management was initiated in evaluation of post radiation necrosis and to differentiate between post radiation necrosis from disease progression [(47)] Feasibility studies that were conducted to assess incorporation of MRS as a standard preoperative MR examination showed that 1H-MRS imaging may be useful for guiding stereotactic biopsies [(48)]

Studies to charecterise serial response of recurrent gliomas to GK(Gamma Knife)-SRS using MR imaging and MRS showed good correlation between areas of spectral abnormalities and subsequent radiologic recurrence and prompted routine usage of MRS in planning for SRS [49]

Importance of various MRS metabolites[50]

Metabolites in brain are in millimolar concentrations and therefore it is very essential to suppress the signals from water. Small voxels and long acquisition times are required to produce low signal to noise ratio.

Absolute quantification of these metabolites is very difficult and hence relative concentration of each using ratio of one another is considered.

Water suppressed localised proton MR spectra of a healthy human reveals four major resonances –

Choline at 3.2 ppm – is derived from membrane phospholipids and high levels associated in brain tumors are due to increased cellular density.

Creatine at 3 ppm – has relatively constant concentration throughout the brain and usually taken as internal standard to which other metabolites are normalised.

NAA at 2 ppm – Is the most important signal in measurement of pathological processes of brain. Mainly seen in mature neurons and is used as neuronal marker in mature human brain.

Lactate at 1.3 ppm – Is the end product of glycolysis and accumulated when oxidative metabolism is unable to meet energy requirements

Metabolite	Chemical shift of mainpeak (ppm)	Description
Cho	3.2	Cho'' includes contributions from choline, phosphocholine, glycerophosphocholine and other trimethylamines.These metabolites are involved in cellmembrane lipid synthesis and breakdown, and are also affected by signalling pathways that can be upregulated in tumours. Since 9 magneticallyequivalentprotons contribute to this peak, relativelylow concentrations produce a measurable signal
Cr	3.02	''Cr'' includes creatine and phosphocreatine, which areboth involved in energy metabolism
Lactate	1.33	Lactate is a product of anaerobic glycolysis, a further aspect of energy metabolism, often being found in necrotic areas
Lipids	1.3	Often found in necrotic regions. This can be detected in brain using shorter echo time acquisitions. Understood to be an essential ingredient for cell growth, an osmolite, and

		a storage form of glucose
NAA	2.01	NAA is considered to be a neuronal marker, so only present in brain
Citrate	2.6	This is synthesized and accumulated by normal prostate epithelial tissue

Single Voxel Magnetic resonance spectroscopy is a non invasive method to characterise the metabolic patterns of various primary brain tumors. Each tumor type has a particular metabolic pattern and this implies whether the tumor type is rapidly proliferating or slowly growing. Studies were conducted in which histological grade on biopsy from these metabolically abnormal areas was found to be correlating with the type of metabolic abnormality in MRS [(51)] Rapidly growing tumors (Anaplastic astrocytoma and Glioblastoma) have increased choline-creatine ratio, reduced NAA-Creatine ratio and increased lactate level.

Single voxel MRS gives metabolic information within a region rapidly it does not address spatial heterogeneity and cannot define spatial extent of lesion.Therefore for treatment planning process wherein spatial extent of lesion is very essential - a multivoxel MRS is necessary [(52)]

Initial studies that confirmed clinical implemetation of MRS were performed in 1.5T MR scanners. 3T scanners provide higher signal to noise ratio with increase in spatial and temporal resolutions and better spectral resolution compared to 1.5T MR scanners. This increases sensitivity of metabolic detection[(53)]

Extent of surgical resection has great impact on survival of patients with GBM. There are anatomic, functional limitations to achieve maximal safe resection and moreover many studies have shown regrowth of tumors between surgery and initiation of RT. It is very essential to have a pre RT MRS for assessing such residual / regrowth which is preferably done within 48 hrs of surgery[54]

Studies done with H1 MRS with chemical shift imaging method showed that this particular imaging modality could be used to differentiate between delayed radiation necrosis and tumor progression. They also observed that serial MRS examinations yielded consistent interpretations over time and at different locations in the developing lesions [55]

3I.Magnetic Resonance Spectroscopy in RT planning

A cooperative group of 15 institutions studied the feasibility of utilising Magnetic Resonance Spectroscopy (MRS) for charecterising brain tumors and concluded that the information of metabolic alteration provided by MRS was reliable and it corresponds to the grade of the tumor [56]

Series of studies done on 3D MRSI showed that in patients with High Grade Gliomas, Choline peaks corresponded with the tumor density and this finding was confirmed with stereotactic biopsy done prior to definitive treatment [57]

Effect of MRS data on radiotherapy planning was first explored by Graves et al who utilised the data for planning gamma knife based stereotactic radiosurgery in patients with recurrent gliomas .They concluded that the data provided by MRSI improves planning and treatment process of glioma patients [58] Following this study MRS was routinely used for all planning process of all patients for gamma knife based stereotactic radiosurgery

Andrea Prizkall et al studied the role of MRS in treatment planning of High Grade Gliomas and concluded that use of MRS would reduce the radiation volume and has a potential to improve control while reducing the complications [(59)]

Rafal Tarnawski et al studied the importance of various metabilites in post op MRS in patients with HGG. They found out the correlation with increase in lac/NAA and survival and observed that in patients with areas of post op brain tissue showing a ratio of more than 2, one year survival was 20% when compared to those with ratio of less than 2 in whom the survival was 85% [(60)]

Andrea Pirzkall et al studied the usage of MRS in post op scans in high grade gliomas prior to radiotherapy planning to assess the extent of residual disease and found out that non uniform margins could be used to define the extent of tumor cell infiltration rather than the use of unifrom margins [(61)]

McKnight et al utilised MRS for defining the exact tumor extensions beyond that defined by MRI. The study showed that High Grade Glioma on imaging is a continuous spectrum of disease progression and the tight compartmental differentiation between low grade and high grade areas was not possible on routine imaging and required higher imaging modalities like MRS [(62)] They also stated that Choline NAA index (CNI) can be used to help identify metabolically active tumor within and beyond the contrast enhancing lesion that appears on MRI .Wide range of CNI that exist throughout the lesion shows various grades of disease that exist in the lesion and it guides as a tool to target the region with highest histological grade . These features have been shown to help in utilising CNI as an in vivo parameter for directing biopsies and focal conformal radiotherapy in non enhancing areas of gliomas.

McKnight et al defined an Abnormality Index (AI) of choline NAA index and defined that all the areas with CNI of more than 2 was the lowest value that showed disease activity in 95% of the patient with HGG[63] In view of this Pirzkall et al defined and evaluated specifically AI levels of 2, 3, and 4 [64]

Narayana et al utilised MRS in radiotherapy treatment planning and found that post contrast T1W images overestimated the GTV as defined by Choline-Creatine ratio (CCI) of 3 by 40% and the T2W imaging overestimated CTV as defined by CCI of 1 by 30 % indicating over treatment of normal brain tissue [65]

Volume delineation with MRS in various studies was done taking different metabolite ratios into consideration. Few studies (Narayana et al) considered the ratio of Choline to Creatine into consideration and all those areas with CCI of >/= 3 was taken into a high risk area and those with lesser values of 2 and 1 were defined as intermediate and low risk volumes respectively. Others (Prizkall et al) took Choline to NAA ratio of 4 as high risk area and other areas with lesser CNI of 3 and 2 as intermediate and low risk areas respectively and all areas with CNI of less than or equal to 1 as normal.

In our study the peak values of CNI and CCI both were considered for volume delineation.

MRS Based Volumes as follows:

CNI – Choline/NAA index; CCI- Choline Creatine Index

GTV: CNI > 3 or CNI >1.5 but <3 and CCI of >1.5

PTV: CNI<1.5 and CCI<1.5

OAR: CNI =/< 1.

4. MATERIALS AND METHODS

Setting of the study:

Study was conducted in Between July 2011 to November 2011 in the departments of Radiation Oncology unit II, Neuro Surgery and Radiology at the Christian Medical College, Vellore.The study was approved by the Institution Review Board (IRB) and was cleared by the ethical committee of the Christian Medical College, Vellore.

A total of ten patients were included in the study. All patients with suspicious High Grade Gliomas on the radiological scans were included in the study. All patients were given information sheet about the study in their own language and were also explained about the study in detail.They were included into the study after taking the inclusion and exclusion criteria into consideration and after taking an informed consent that was provided to them in their own language.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

Patients > 18 yrs

Performance status KPS > 70

High Grade Glioma histology

Exclusion criteria:

Patients with age < 18 yrs

Pregnancy

Patients with low grade glioma histology

Patients with pacemakers, cochlear implants, some types of aneurysm clips and implanted drug infusion devices.

Patients with other devices, such as heart valves, and middle ear prosthesis.

Patients with Claustrophobia

Basic Methodology of the study:

Patients included into the study underwent a preop imaging with MRI and MRS. Later these patients underwent a surgical procedure. Following the surgical intervention, all those who underwent an excision procedure underwent an imaging with MRI and MRS. Once the histological diagnosis was confirmed these patients were planned for local radiation therapy.

Detailed Methodology of the study:

1) Participants: All patients with a radiological diagnosis of High Grade Glioma

2) Enrollment in the study:

All those patients with a radiological suspicion of High Grade Glioma were given information sheet in their language. For those patients who met the inclusion and the exclusion criteria, a consent sheet in their language was provided. After obtaining an informed consent, they were enrolled into the study.

3) Preoperative Imaging:

Preoperative imaging consisted of Magnetic resonance Imaging and Magnetic Resonance Spectroscopy.

Magnetic Resonance Imaging:

MR examination was performed in Phillips 3T scanner

Sequences performed include:

T2W images in all the three planes (coronal, sagittal and tranverse)

T2W FLAIR sequences.This was taken as a volumetric series with a slice thickness of

3 mm with 0 mm gap between the slices.

T1W pre contrast sequences.

Post Gadolinium T1W sequnces in all the three planes.

Total duration of MRI was about 45 minutes

Machine parameters for MRI FLAIR squences are as follows:

Parameter	Value
TR/TI	11000/2800
Act TE	120
ACQ matrix	256x132
ACQ voxel	0.90x138x5.00
Recon Voxel	0.45x0.45x5.00

Magnetic Resonance Spectroscopy (MRS):

MRS was performed after T2W FLAIR sequences but prior to contrast injection.Multivoxel MRS was performed with aneffective matrix size of 6x6 with each voxel about 1 cm.

Machine parameters for MRS are as follows:

Parameter	Routine MRS	Study MRS
ACQ(acquisition)matrix	15x12	15X12
AP:RL	230X184	230X184
Voxel size	15x15mm	10x10mm
Recon Voxel	10mm	10mm
TR/TE	2000/58ms	852/58ms
ST	15 mm	15 mm
Sense	yes	yes
Suppression	1024	1024
Spell BW	2400	2400
M reduction	1	4
P reduction	1	1

PRESS box was positioned so that it extended well beyond the suspected disease to include the normal brain in the contralateral hemisphere even if it meant that the suspected lesion could not be completely encompassed. It was placed to avoid areas with subcutaneous lipid with varying magnetic susceptibility that might compromise the quality of spectra.

Similar multivoxel MRS images were taken at multiple levels which included – the enhancing component on FLAIR sequences, images with edema alone and those with normal brain only.

The total duration of MRS study was about 20 minutes.

Peak parameters for choline, creatine, NAA, lipid and lactate and then the choline-creatine index (CCI) and Choline-NAA index (CNI) was defined on a voxel by voxel basis.

The MRI T2W FLAIR imageswere transferred to Oncentra treatment planning system via PACS which is the central work station of department of radiology. As the MRS images could not be converted to gray scale for fusion with MRI, these images were transferred to a compact disc separately for each patient and the images were directly compared for volume delineation.

4) Surgical Procedure:

Patients, who underwent pre operative imaging, later were planned for surgical excision. The type of excision varied between patients.

Depending on extent of resection, it was either a gross total resectionwhich involvedcomplete surgical extirpation (90- 98%) of the lesion or a partial excision which involved removal of surgically accessible portion of the lesion without excision of that part of the lesion near the eloquent areas.Stereotactic Biopsy was performed in those patients for histological confirmation of the type of glioma in whom a surgical excision was not feasible.

5) Post operative imaging:

All patients who had either a partial excision or a complete excision underwent post operative imaging with MRI and MRS preferably within 48 hours of surgical procedure. In those patients with low performance status, a post op MRI and MRS was performed along with radiation planning process.

The MRI and MRS protocol in the post op setting was similar to that perfromed in the pre op setting.

Post operative imaging was not done in those who did not undergo an excision procedure and those who underwent only a biopsy procedure.

6) Radiation therapy planning process and volume delineation:

Immobilisation: This was done as per the routine protocol.Immobilisation was done with ray cast and a pseudo isocentre was placed over the anatomical bony landmarks of the skull. Following this a planning CT scan was performed with the immobilisation.

Planning CT scan: It was performed in a Siemens spiral CT scanner that had facilities for a planning CT scan including a flat couch, wide bore gantry (70 cm) and laser for matching the isocentre. A scout topogram of the desired image level was taken and after verification of the isocentre, 80 ml of iodinated contrast was administered intravenously prior to scan. Images were acquired from vertex to lower border of the second cervical vertebra. The slice thickness was 3 mm with no gap between each slice.

All the preoperative and post-operative MRI and CT images were transferred to the Oncentra planning software through the PACS software. As the MRS images could not be converted to gray scale and fusion with MRI images was not possible, the images were transferred to a compact disc in DICOM (Digital Imaging and Communication Media) format for further usage.

In those patients who were not willing to undergo radiation therapy, the immediate post op CT scan that was done to assess residual disease was transferred to Oncentra planning system for volume delineation. These CT images were taken with a slice width of 5 mm with 0 mm gap between the slices.

These CT images were transferred to Oncentra planning software. CT images were co registered with MRI images by the automated software system in the Oncentra and the routine volume delineation was performed as per the RTOG guidelines. MRS images were not directly fused with the MRI or CT images (as they were not converted into gray scale) and the volume delineation was performed manually by direct comparison.

Volume definition with CT and MRI were done as per the RTOG guidelines as below:

GTV=gross tumor volume; PTV = planning target volume

GTV - enhancement seen on T2W FLAIR images (contrast enhancing lesion and surrounding edema)

PTV - GTV plus 2.5 cm uniform margin

7) MRS based volume delineation:

Peak parameters for choline, creatine, NAA, lipid and lactate was estimated on a voxel by voxel basis and the choline-creatine index (CCI) and Choline-NAA index (CNI) was defined. Once the peak parameters and ratio of various metabolites are defined on the MRS, the following risk volumes were defined.

Volume delineation with MRS in various studies was done taking different metabolite ratios into consideration. Some studies (Narayana et al) considered the ratio of Choline to Creatine into consideration and all those areas with CCI of >/= 3 was taken into a high risk area and those with lesser values of 2 and 1 were defined as intermediate and low risk volumes respectively.While others (Prizkall et al) took Choline NAA index of 4 as high risk area and other areas with lesser CNI of 3 and 2 as intermediate and low risk areas respectively and all areas with CNI of less than or equal to 1 as normal.

In our study it was observed that the peak values of CNI and CCI were at a lower range when compared to that of reference studies and hence we decided to take both the ratios into consideration for volume delineation.

MRS Based Volumes were defined as follows:

CNI – Choline/NAA index; CCI- Choline Creatine Index

Multivoxel MRS was done at various levels on the FLAIR sequences. These included enhancing component that contains ring enhancing lesion and surrounding enhancing edema, those regions with non enhancing edema alone and those areas with normal brain.At these image levels, areas with Choline Creatine Index of more than 1.5, Choline NAA Index (CNI) of more than 3, CNI of 1.5 to 3 and CNI of less than 1 was defined. These were defined on a voxel by voxel basis.As per the studies mentioned previously,areas with CNI, or abnormality index of 2 were found to have disease activity in more than 95% of cases studied and was also found that CNI was more specific compared to other metabolite ratios. CCI was found to be high in areas with necrosis.

Areas with CNI more than 3 and those areas with CNI between 1.5 and 3 were defined on a voxel by voxel basis. The parameters defined at these image levels on which MRS was performed, was extrapolated to other images of FLAIR MRI on which MRS was not performed. The three dimentional MRS volume defining the high risk area encompassing CNI of more than 3and intermediate risk area encompassing CNI of 1.5 to 3 was compared with the volume defined by post Gadolinium T1W and T2W FLAIR sequences. Disjoint and conjoint volumes were then defined by comparing the volumes defined by the MRI alone and combined MRI & MRS. Similar comparison was made between volumes defined by areas encompassing CNI less than 1.5 and non enhancing edematous component on FLAIR MRI.

Following comparisons were made

MRI VOLUMES	**MRS VOLUMES**
T1W GTV (Enhancing component on T1W image)	CNI of more than 3
T2W GTV (Enhancing component on T2W FLAIR image)	CNI of 1.5 to 3 and CCI more than 1.5
PTV (GTV + 2.5 cm uniform margin)	CNI less than 1.5 + 5 mm

MRS analysis for volume delineation was done later than the MRI and hence the MRS data was not utilised for treatment of these patients.Treatment planning of all patients were done as per CT-MRI based volume delineation.

In our study, 22 patients with radiological suspicion of Glioma were screened for the protocol. Out of 22 patients, 6 patients did not have high grade features on MRI, 4 patients underwent biopsy that had other histologies than HGG. 2 patients had deterioration in the general condition and could not be included in the study. Ten patients who were eligible by inclusion and exclusion criteria out of 22 participated in this study.

RESULTS

Table 1: Patient Characteristics:

AGE (years)	N = 10
Range	22 - 60
< 30	2
30 – 40	3
40 – 50	3
50 – 60	2
SEX	
Male	7
Female	3
PERFORMANCE STATUS – ECOG	
1	4
2	6
PRESENTING SYMPTOM Headache	5
Neurological Deficits	2
Seizure	2
Visual Changes	1

The table 1 shows the patient characteristics

Majority of the patients were in the age group of 30- 65 years (N=8). Only 2 patients were in the age group of less than 30 years.

Male to female ratio was 2.3:1.

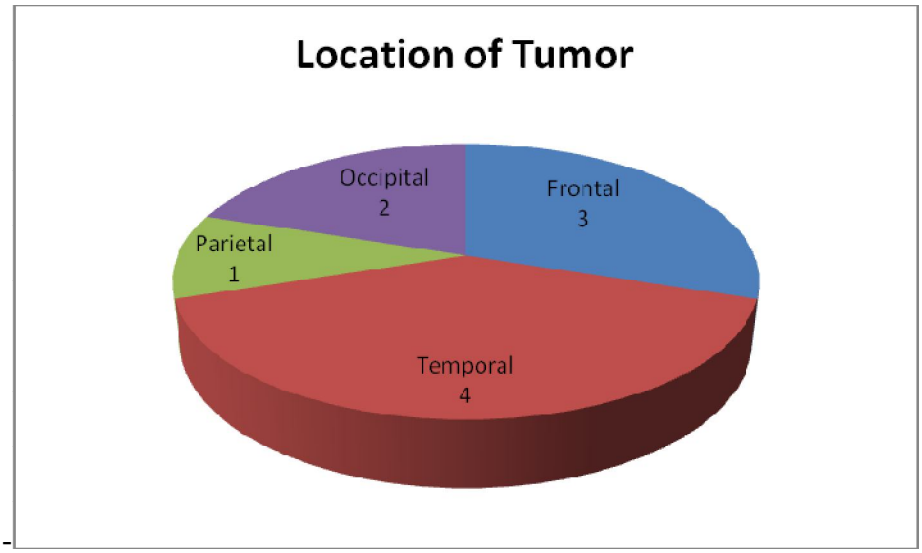
Performance status in most of the patients was ECOG 2 (N= 6) and in the rest of the patients it was ECOG 1 (N=4)

Headache was the main presenting complaint and was present in 5 patients followed by neurological deficit in 2 patients, seizure in 2 patients and visual changes in one patient.

Table 2: Location of the tumor

LOCATION OF TUMOR	
Frontal	3
Temporal	4
Parietal	1
Occipital	2

Graph 1: **Location of the tumor**



Tumor was seen in in temporal lobe in 4 patients, frontal area in 3 patients, occipital cortex in 2 patients followed by parietal lobe in one patient.

Table 3: Type of Surgery

TYPE OF SURGERY	
Stereotactic Biopsy	2
Open Biopsy	1
Partial Excision	6
Radical Excision	1

Graph 2: Type of Surgery

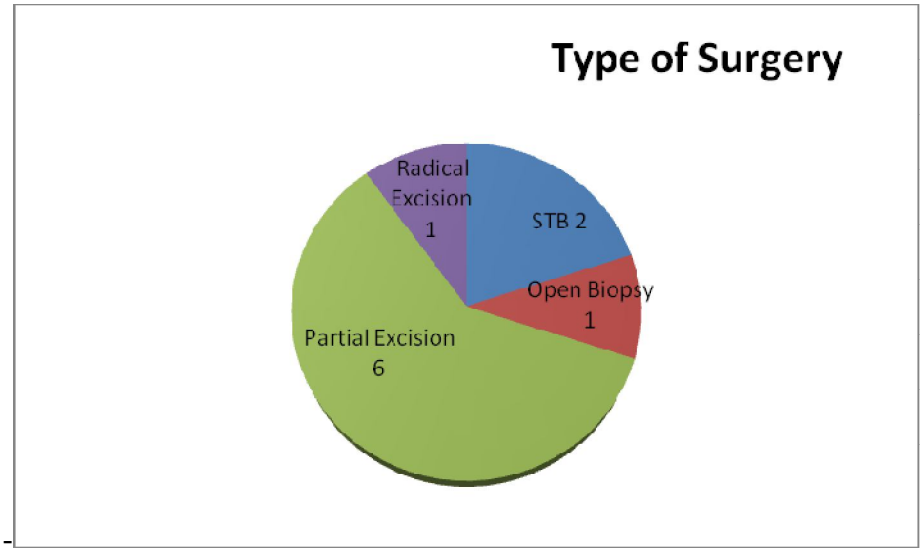


Table 3 and Graph 2 shows that 7 out of 10 patients had underwent surgery (6 had partial excision and 1 had radical excision) and 3 patients had biopsy only (2 had sterotactic biopsy and I had open biopsy)

Table 4: Histological characteristics

HISTOLOGY	
Anaplastic astrocytoma	3
Anaplastic Oligodendroglioma	1
GlioblastomaMultiforme	5
Gliosarcoma	1

Graph 3: Histological characteristics

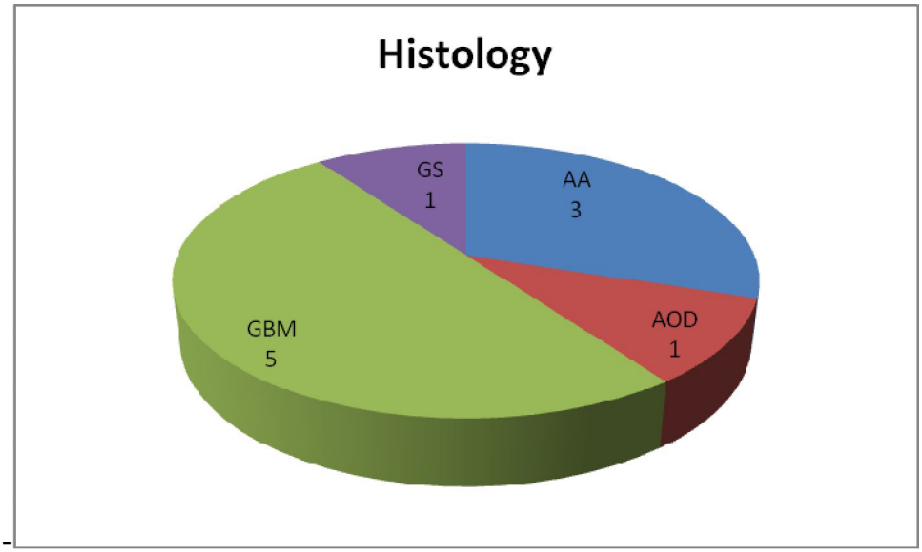


Table 4 and Graph 3 shows that 5 patients had histology of Glioblastoma multiforme and this was followed 3 patients with anaplastic astrocytoma and histology of anaplastic oligodendroglioma and gliosarcoma in one patient each.

Table 5: Comparison of GTV defined by T1, T2 FLAIR and MRS (CNI 1.5 – 3+ CCI >1.5)

S no.	T1 GTV (cc)	T2W GTV FLAIR (cc)	GTV MRS (CNI 1.5 - 3) (cc)
1	53.6	253.6	147.8
2	17.6	120.6	34.1
3	90.2	241.4	173.3
4	85.5	173.1	88.8
5	31.4	89.8	53.2
6	6.1	12.6	12.9
7	144.5	175.2	15.4
8	66.5	251.7	60
9	5.1	10	8.4
10	28.9	65.1	82.9

Table 5 shows the comparison of volumes defined by T1 / T2 MRI series and MRS

When T1 MRI defined GTV was compared with GTV defined by intermediate risk area MRS (CNI 1.5-3), it was found that 8 out of 10 patients had larger volume on MRS. The remaining two patients had more volume on T1 MRI. When T2 MRI defined GTV was compared with the GTV defined by intermediate risk area MRS (CNI 1.5-3), it showed that 8 out of 10 patients had less volume on MRS when compared to T2 MRI. In remaining 2 patients the volume of GTV delineated with MRS guidance was more. (One patient was diagnosed to have GBM and the other was anaplastic astrocytoma).

The same is depicted in Graph 4

Graph 4: Comparison of GTV defined by T1, T2 FLAIR and MRS (CNI 1.5 – 3+ CCI >1.5)

-

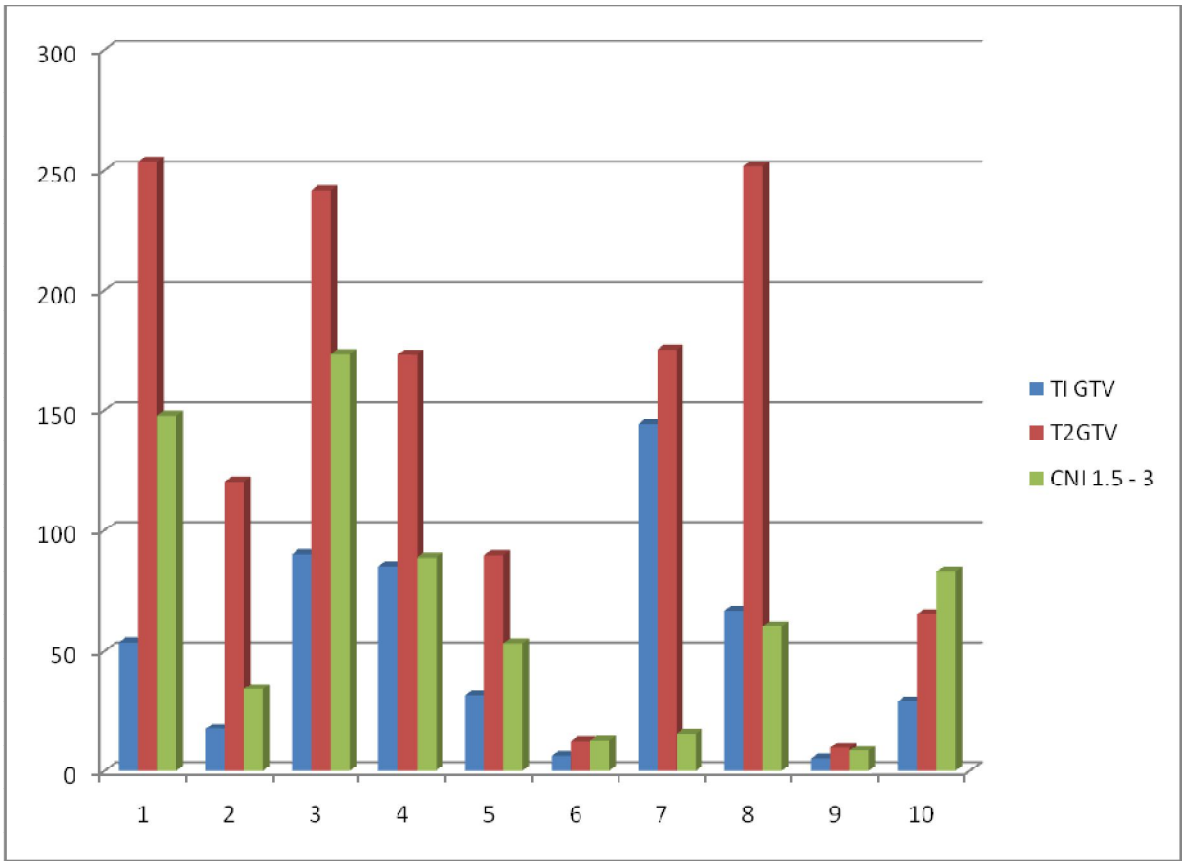


Table 6: Comparison of GTV defined by MRI TI (cc) to the MRS defined GTV volume (cc)[High risk area (CNI >3)]

S No	T1GTV MRI (in cc)	GTV MRS (CNI>3) in cc	% of difference (T1 GTV- MRS GTV / T1 GTV)*100
1	53.6	20.5	61%
2	17.6	14.4	18%
3	90.2	52.9	41%
4	85.5	0	100%
5	31.4	0	100%
6	144.5	0	100%
7	6.1	0	100%
8	66.5	0	100%
9	5.1	0	100%
10	28.9	20.1	30%

Graph 5: Comparison of GTV defined by MRI TI (cc) to the MRS defined GTV volume(cc)[High risk area (CNI >3)]

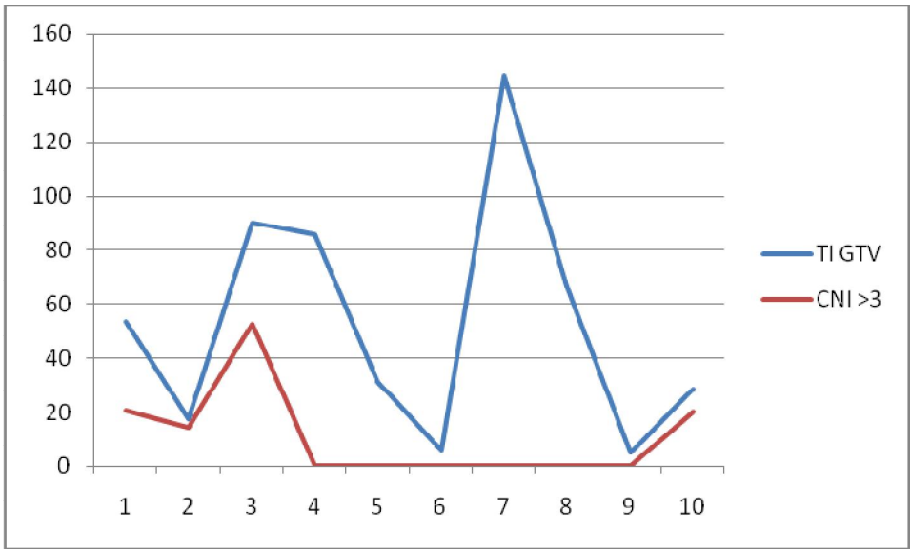


Table 6 and Graph 5 show the difference in the GTV volumes defined by T1 MRI and MRS (High risk area CNI> 3).

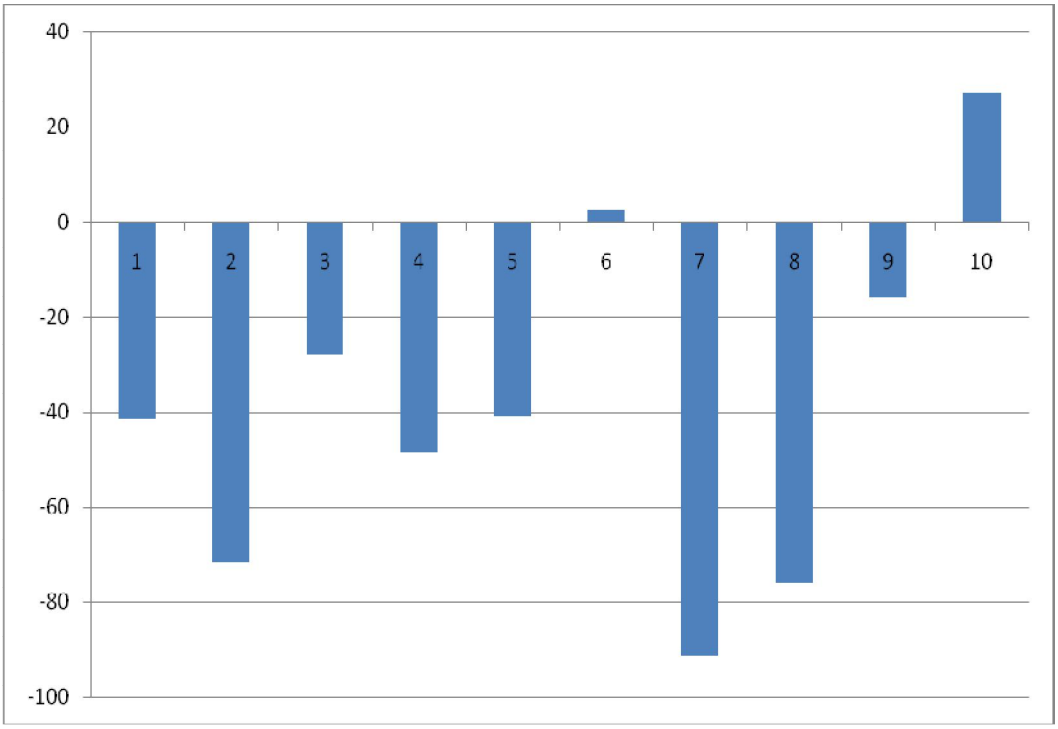
Four patients had high risk area on the MRS as shown by CNI ratio of more than 3. When comparison was made between the GTV defined by contrast enhancing lesion defined on T1 MRI and the GTV defined by MRS >3 in these four patients, it was found that MRI defined GTV was more by an average of 37%, the range being 18%-61%. Among the remaining 6 patients, there was no MRS defined high risk area which means that the T1MRI defined GTV was more by 100%.

Table 7: Comparison of T2 FLAIR GTV volume (cc) to MRS defined GTV Volume (cc) [Intermediate Risk area (CNI 1.5-3)]

Sl No	T2GTV MRI (cc)	Intermediate Risk Area MRS (CNI 1.5-3) (cc)	% difference in volumes (T2 GTV- MRS IRA / T2 GTV)*100
1	253.6	147.8	41%
2	120.6	34.1	71%
3	241.4	173.3	17%
4	173.1	88.8	48%
5	89.8	53.2	40%
6	175.2	154.4	12%
7	12.6	12.9	**- 2%**
8	251.7	60	76%
9	10	8.4	16%
10	65.1	82.9	**- 27%**

Table 7 shows comparison of GTV defined by T2W FLAIR MRI and MRS defined by intermediate risk area (CNI ratio of 1.5-3). It shows that GTV defined by T2 MRI was more in 8/10 patients, than the corresponding MRS defined intermediate risk area by an average of 40% (Range 12% - 76%). In the remaining 2 patients MRS defined GTV was more by 2% and 27% respectively.

Graph 6 : Comparison of T2 FLAIR GTV volume (cc) to MRS defined GTV Volume [Intermediate Risk area (CNI 1.5-3)]



The above graph depicts that in 8 out of 10 patients GTV defined by T2 MRI was more in 8/10 patients, than the corresponding MRS defined intermediate risk area

Two patients had increase in GTV volume as defined by intermediate risk MRS. One patient was diagnosed to have GBM and the other was anaplastic astrocytoma . MRS in these two patients demonstrated metabolic activity beyond the GTV that was seen on the MRI and in one of these patients this was situated over the brain stem.

Table 8: Comparison of PTV volume in MRI (GTV T2 FLAIR+2.5 cm) and PTV in MRS (CNI< 1.5+ 5 mm)

S. No	PTV MRI (cc) (GTV T2 FLAIR+2.5 cm)	PTV MRS (cc) (CNI <1.5 + 5 mm)	% of difference (PTV MRI - PTV MRS / PTVMRI)*100
1	1029	534.1	92
2	730.2	264.2	63
3	1191	532.5	55
4	821.6	417.3	82
5	642.8	250.9	60
6	729.7	432	40
7	245.8	109.6	55
8	771.7	417.8	45
9	159.3	94.4	40
10	608	332.4	45

Table 8 and Graph 7 shows the comparison between PTV defined by MRI and MRS. PTV defined by MRI was more in all the patients when compared to PTV defined by MRS. This was on an average 57% (range 40%- 92%).

For the two patients who had larger GTV on MRS was found have while generating PTV from these GTV, it was found that the PTV MRI enclosed the MRS and there were no disjoint volumes in these two patients.

Graph 7: Comparison of PTV volume in MRI (GTV T2 FLAIR+2.5 cm) and PTV in MRS (CNI< 1.5+ 5 mm)

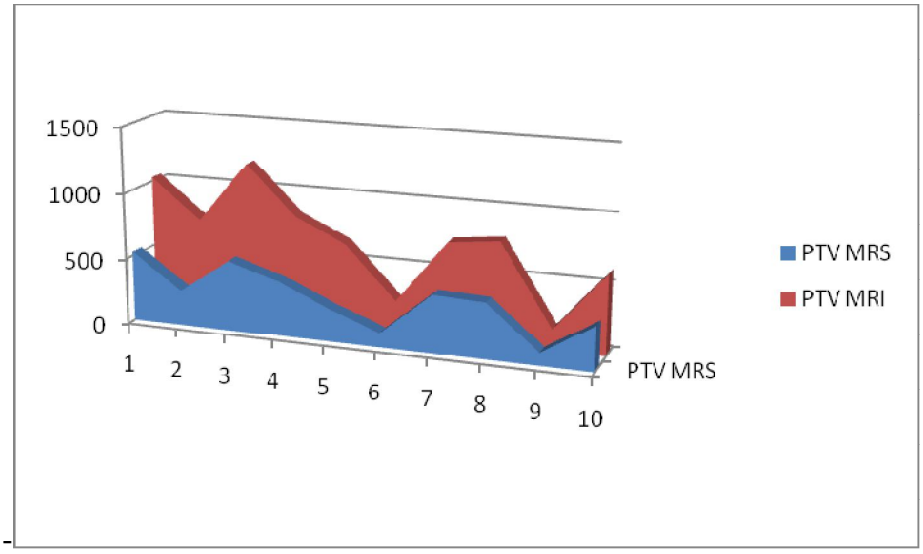


Table 9: Disjoint GTV volumes as defined by T1 MRI and GTV defined by MRS (CNI 1.5-3)

Patient	GTV T1 MRI (cc)	GTV MRS CNI(1.5- 3) (cc)	T1 GTV BEYOND MRS (CNI 1.5-3) (cc)	MRS (CNI 1.5-3) BEYOND T1 MRI GTV (cc)
1	53.6	147.8	16.2	111
2	17.6	34.1	1	16.1
3	90.2	173.3	8.1	94.2
4	85.5	88.8	84.5	3.4
5	31.4	53.2	4.8	26.2
6	6.1	12.9	1	5.9
7	144.5	15.4	1.7	6.3
8	66.5	60	8.8	3.4
9	5.1	8.4	1	3
10	28.9	82.9	1	52.7

Graph 8: Disjoint GTV volumes as defined by T1 MRI and GTV defined by MRS (CNI 1.5-3)

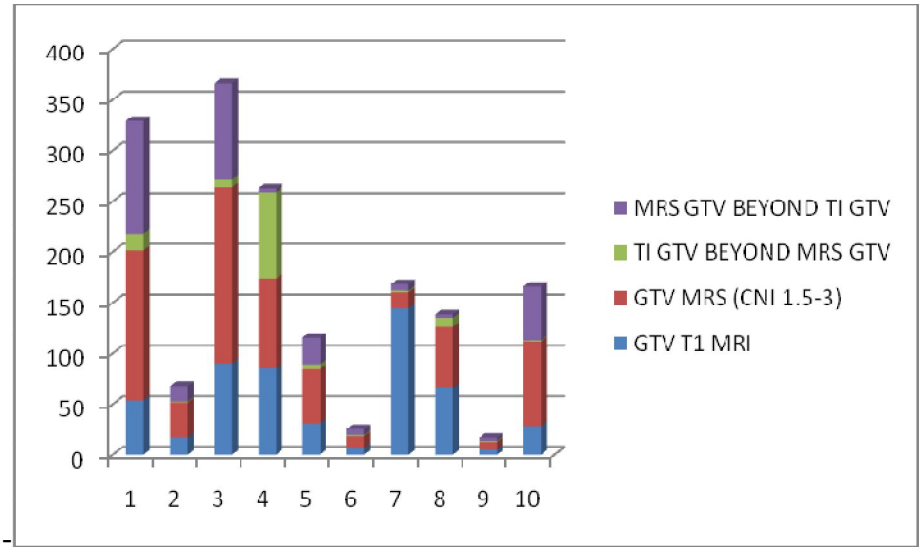
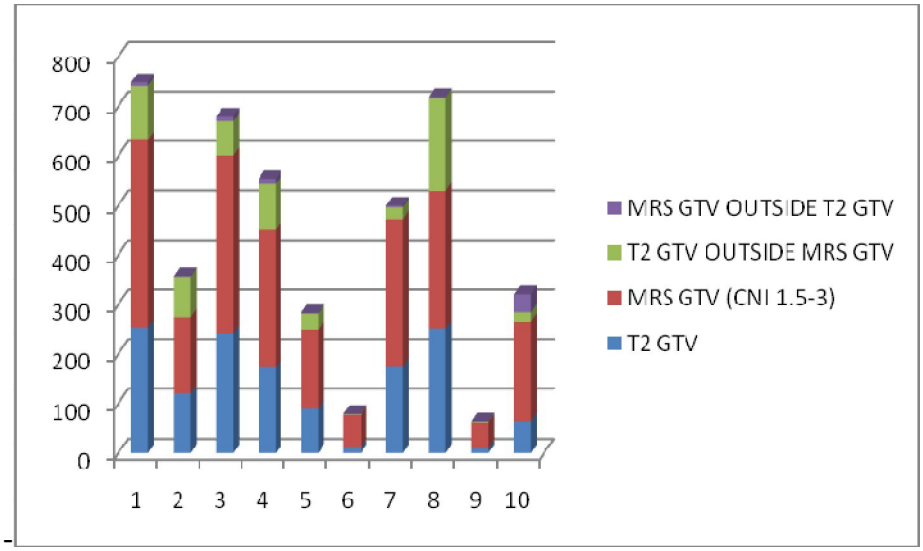


Table 10: Disjoint GTV volumes as defined by T2 MRI and GTV defined by MRS (CNI 1.5-3)

Patient	T2 MRI (cc)	GTV MRS CNI(1.5- 3) (cc)	T2 GTV BEYOND MRS (CNI 1.5-3) (cc)	MRS (CNI 1.5-3) BEYOND T2 MRI GTV (cc)
1	253.6	378.4	109	7.3
2	120.6	154.7	81.9	1
3	241.4	360.3	67.5	10.6
4	173.1	277.6	92	11.2
5	89.8	159.9	32.5	1
6	12.6	65.1	1.3	1
7	175.2	298.6	23.1	3.3
8	251.7	277.4	189.2	1
9	10	53	1.3	1
10	65.1	201.6	18	35.8

Graph 9: Disjoint GTV volumes as defined by T2 MRI and GTV defined by MRS (CNI 1.5-3)



Tables 9 and 10 / Graph 8 and 9

Disjoint MRI and MRS defined Gross Tumor volumes

The tables show the Disjoint volumes of MRI and MRS defined GTV.

When the disjoint volume of GTV defined by T1 MRI and MRS (CNI 1.5- 3) was compared it was found that MRS defined GTV volume extended beyond MRI defined volume in all patients by an average of 32.22 cc and the range was between 3 cc and 111 cc.

Comparison of GTV defined by T1 MRI and MRS (CNI1.5-3) showed that T1 MRI defined GTV was beyond the MRS volume by an average of 13 cc with minimum of 1 cc and maximum of 84.5 cc.

Comparison of GTV defined by T2 MR and MRS CNI 1.5-3 showed that T2 MR defined GTV was beyond the MRS volume by an average of 61.58 cc with minimum of 1.3 cc and maximum of 189 cc.

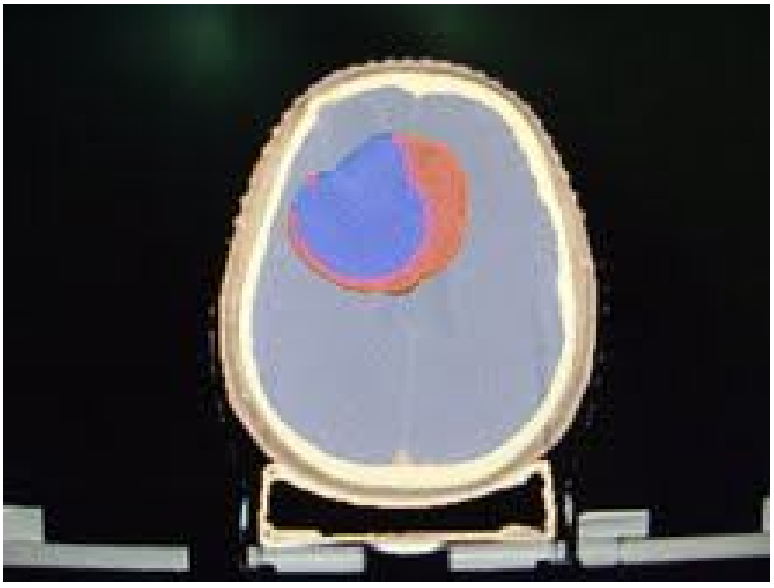
Comparison of GTV defined by T2 MR and MRS CNI 1.5-3 showed that CNI 1.5-3 defined MRS volume was beyond the T2 MRI volume by an average of 7.32 cc with minimum of 1 cc and maximum of 35.8 cc.

When T1 MRI is used for GTV delineation and when compared with MRS defined GTV, an average volume of 32.22 cc of volume of active disease beyond that defined by MRI alone is seen.

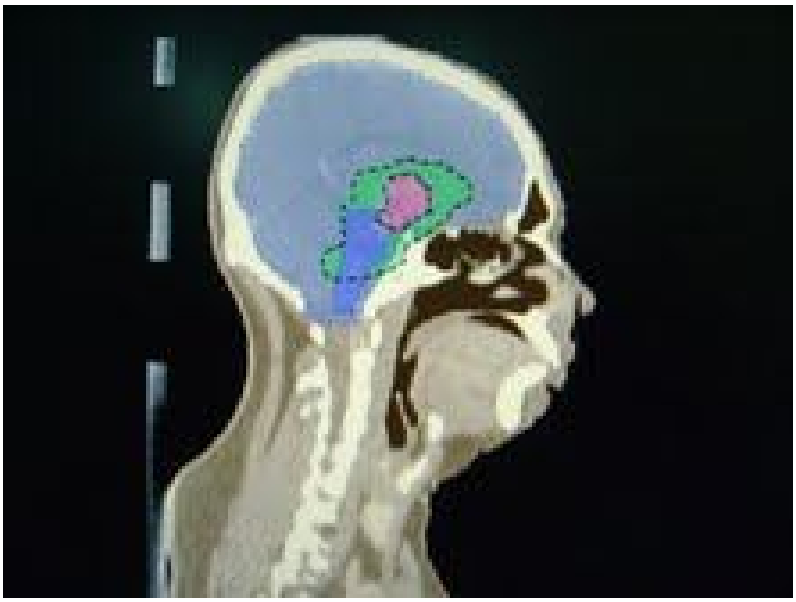
The MRS defined GTV extended beyond the T2W FLAIR volume by an average volume of 7.32 cc (range 1-35.8cc) which is the active disease that has been missed on the MRI. While PTV is generated from these GTV, it was found that the PTV MRI enclosed the MRS and there were no disjoint volumes in these two patients.

Volume of GTV defined by T1 MRI alone that is beyond the MRS defined GTV is in an average of 84.5 cc(Range 1cc - 84.5cc)and when T2W FLAIR was used alone in defining GTV, volume of GTV defined by MRI that was beyond MRS was about 61.58 cc(range 1.3cc -189.2 cc)which indicates that a substantiate amount of normal brain tissue in the irradiated volume. This would lead to increased risk of acute and late toxicities.

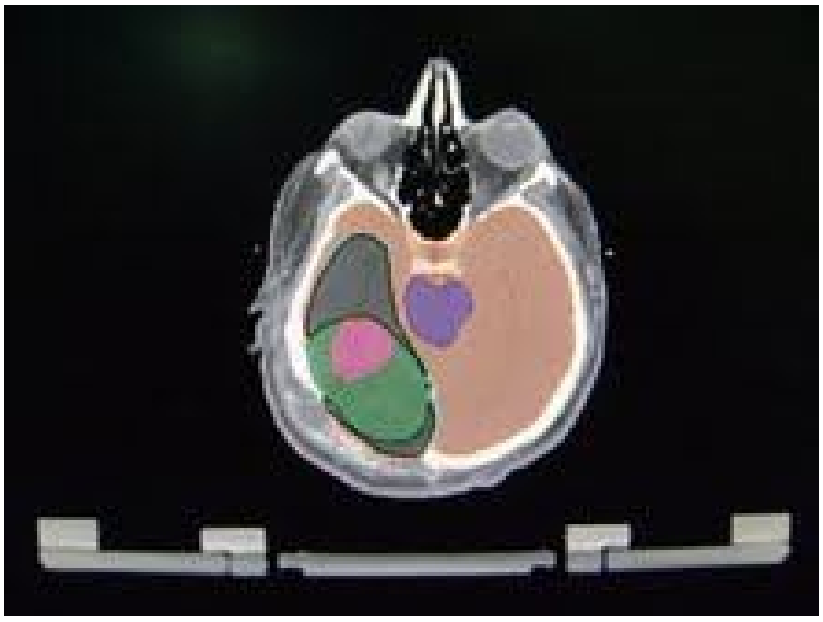
Picture below Depicts the Conjoint T1 GTV Volume (Blue) of MRI in a patient with Glioblastoma that is much less than the corresponding High Risk Area (Brown) in MRS seen as disjoint area



Comparison of T1 GTV Conjoint and Disjoint areas .The picture shows that the MRS defined High Risk area extends beyond the MRI defined T1 GTV especially over the Brainstem area



The picture below shows disjoint area depicting that volume of intermediate risk area (black) on the MRS extends beyond the GTV on FLAIR MRI (green).



The picture below shows the extension of Intermediate risk area in the MRS beyond T2 FLAIR defined volume on MRI – the disjoint volume (dark blue) and the conjoint volume in brown.

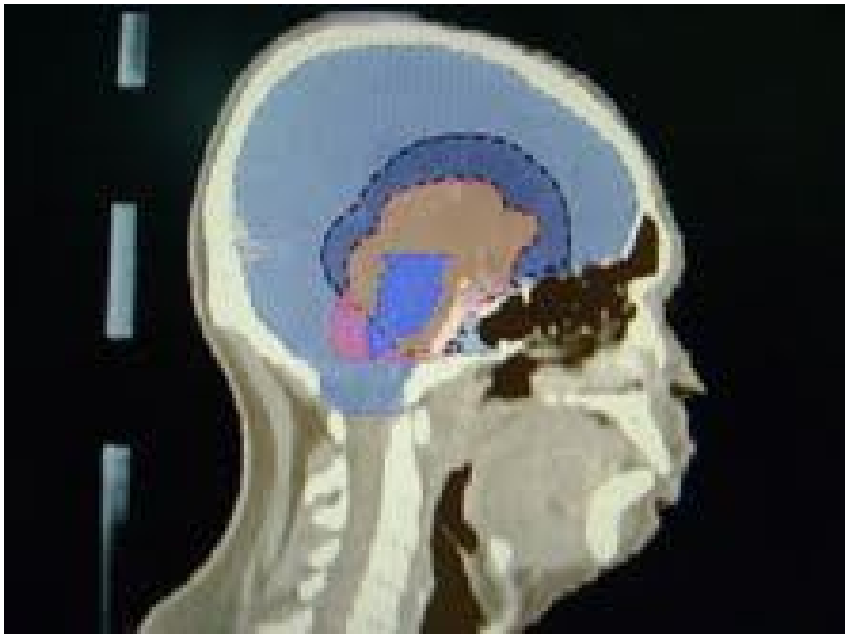


Table 11: Comparison of Conjoint and Disjoint volumes PTV defined by MRI and MRS

Patient	PTV MRI (cc)	PTV MRS (cc)	CONJOINT PTV (cc)	DISJOINT PTV PTV MRI beyond PTV MRS(cc)	DISJOINT PTV PTV MRS beyond PTV MRI(cc)
1	1028.9	534.1	517.4	445.2	NIL
2	730.2	264.2	260.8	473	NIL
3	1191.3	532.5	518.6	623.9	NIL
4	821.6	417.3	394.4	399.6	NIL
5	642.8	250.9	247.4	403.5	NIL
6	245.8	109.6	108.2	134.9	NIL
7	729.7	432	416.6	300.7	NIL
8	771.7	417.8	393.5	364.6	NIL
9	159.3	94.4	92.8	63.2	NIL
10	608	332.4	260.5	279.2	NIL

Graph 10 Comparison of Conjoint and Disjoint volumes PTV defined by MRI and MRS

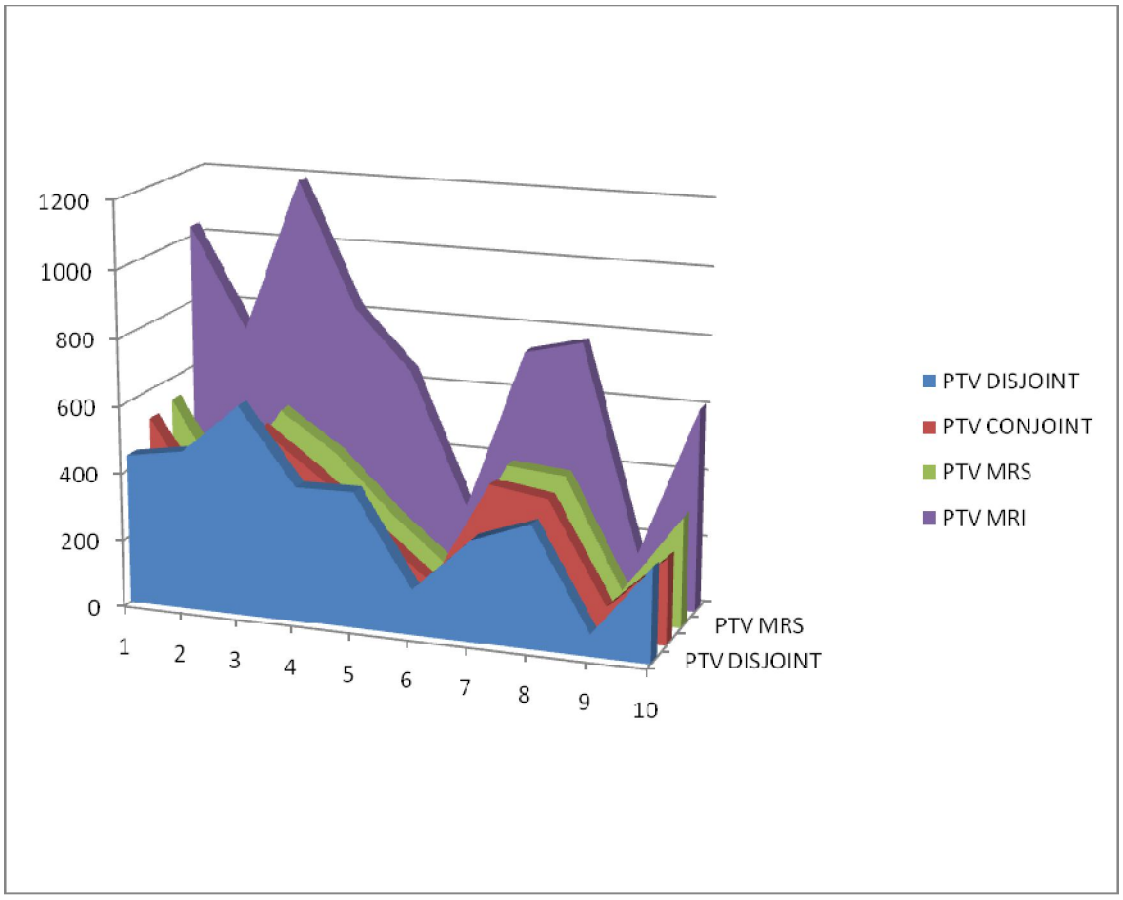
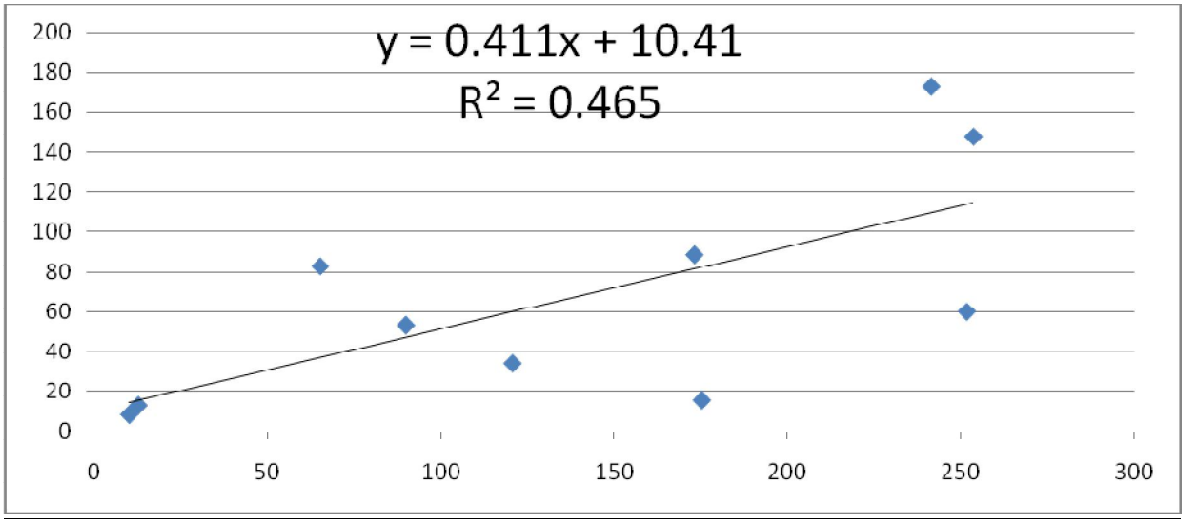


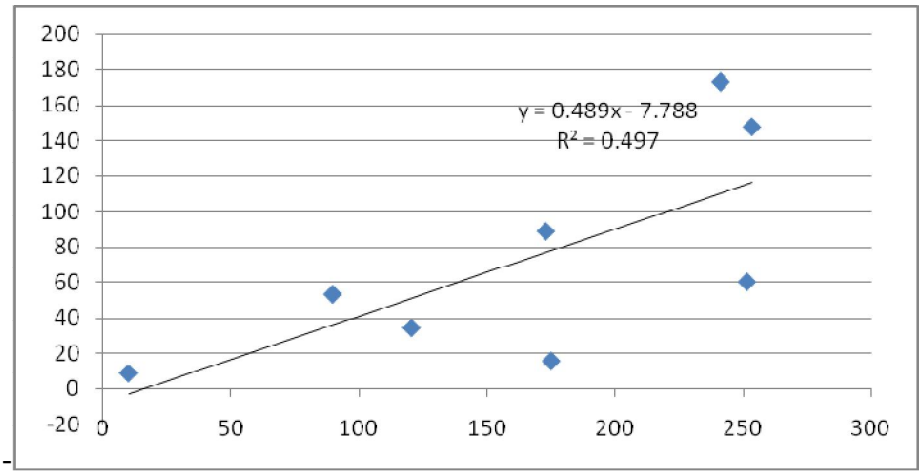
Table 11 and graph 10 show that the PTV volume defined by MRI was beyond the PTV volume volume defined by MRS in all the patients. The average disjoint volume between PTV MRI and MRS was 348.78 cc and the range was 63.2 cc to 623.9 cc.

There was no disjoint PTV MRS beyond the PTV MRI volume for any patient which implies that PTV volume of all the patients defined by MRI was encompassing PTV MRS for all the patients.

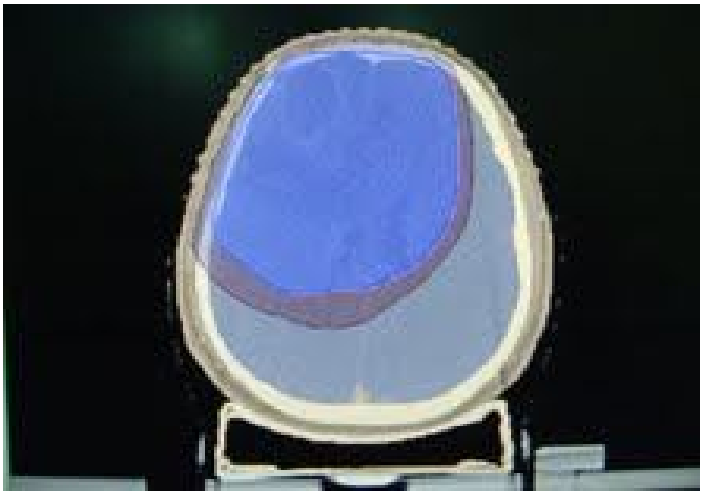
Graph 11 shows the pattern of regression of values beyond the mean value of GTV defined by MRS CNI (1.5-3). The graph depicts regression pattern for all the 10 patients.



Graph 12 shows the regression pattern excluding those patients in whom the MRS defined GTV was more than the T2 MRI defined GTV

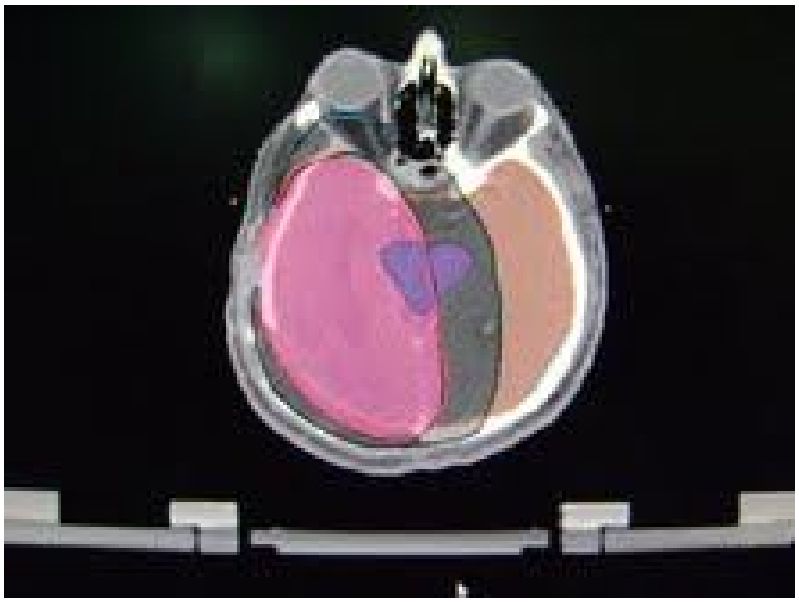


Picture Below shows the Conjoint PTV Volume (Blue) and Disjoint PTV volume (Brown) in a patient with Glioblastoma



The pictures shows the conjoint and disjoint PTV volumes of MRI and MRS

MRI defined PTV (dark) extends into the orbit whereas the extension is not seen in the PTV defined by MRS.



Statistical Analysis:

Statistical tool used was Wilcoxon Signed Rank test that tests the significance of two variables that are obtained by two different modalities.

1. Significance of Comparison of PTV defined by MRI versus PTV defined by MRS

	PTV MRI	PTV MRSI
N	10	10
Mean	692.93	338.52
SD	312.9	157.17
Min	159.3	94.4
Max	1191.3	534.1

Wilcoxon signed-rank test:

Adjusted variance = 96.25

z = 2.803

Prob> |z| = 0.0051

Test 1 shows the significance when PTV is defined by MRI and MRS. The Probability value is 0.0051 which shows that there is statistical significance between the two modalities for definition of PTV.

2. Significance of Comparison of GTV defined by MRI versus GTV defined by MRI

	GTV T2W FLAIR MRI	GTV MRSI
N	10	10
Mean	139.31	81.58
SD	94.06	59.39
Min	10	8.4
Max	253.6	173.3

Wilcoxon signed-rank test: Adjusted variance = 96.25z = 2.395

Prob> |z| = 0.0166

Test of significance for GTV shows a p value of 0.0166 which means that there is statistical significance between the two modalities for definition of GTV.

DISCUSSION

Survival of patients with High Grade Gliomas has been dismal in spite of advancements in treatment techniques. The optimal treatment of High Grade Gliomas (HGG) is maximal safe resection followed by post-operative chemo irradiation and adjuvant chemotherapy.

Radiation therapy of High Grade Gliomas (HGG) is challenging. Walker et al found out that with maximal safe resection followed by whole brain radiation therapy (WBRT) improved median survival of patients with HGG from 13 weeks with best supportive care to 35 weeks with adjuvant radiation. The total dose in these studies was not more than 45 Gy due to risk of radiation induced toxicity to the brain. The radiation was delivered as whole brain RT. [66]

Hochberg and Pruitt et al comparing whole brain radiotherapy and partial brain external beam radiotherapy (PBRT) and found that there was no statistically significant difference in survival between WBRT and PBRT. (68)Halperein et al did a series of autopsy studies patients of high grade glioma also showed that majority of patients had tumor recurrences around the resection cavity and that inclusion of whole brain in the radiation portal led to radiation induced damage to the normal brain substance.

In the older studies, total dose was limited to 45 Gy or less due to the risk of radiation induced toxicity to the brain. Bleehen et al showed that with addition of extra dose by increasing the dose delivered to the target volume, there was significant improvement in progression free and overall survival from 9 months with 45 Gy and 12 months with 60Gy. (67) In view of this there is a shift towards using better imaging modalities for localization of tumor and better delineation of target volumes. Thornton et al demonstrated the superiority

of conformal plans over the conventional plans for sparing the brain volumes while delivering high doses with conformal radiotherapy (CRT) to the target volume. (69)

Thornton et al showed that radiation volumes that were expanded by 3 cm with CT to define the initial target volume could be reduced to 2 cm outside the T2-MRI defined edematous brain and similarly the boost tumor volume expansion could be reduced to 1cm in all directions. The outcomes analyzed in both groups were similar.

Susan lee et al (1999) studied the patterns of failure after 3DCRT in her quantitative dosimetric study. These patients were treated with CT based RT planning and 3 cm expansion of tumor on CT was used to generate the PTV. Recurrences were defined on the MRI and the images were fused to the palnning CT scan . She showed that recurrences after 3DCRT were inside the RT portal in 89%, marginal in 8% and outside the RT portal in 3 %. (71) With the advent of newer imaging modalities like MRI, the tumor delineation was better. Eventhough MRI was superior to CT in guiding volume delineation, there are some limitations seen with MRI.

Thornton et al, Halperien et at and Byrne et al have reported on the limitations of MRI. They have shown that T1 and T2 MRI may not always correspond to regions of active disease and microscopic disease. Central necrotic tissue seen on T1 MR that may be enhancing in few cases does not always correspond to active disease. T2MRI may show hyper intensity for nonmalignant processes such as inflammation and reactive edema formation and hence makes it difficult to determine tumor extent.

Kelly et al conducted image guided serial biopsies in previously untreated glioma patients and showed that showed tumor cells extending in a variable pattern beyond the enhancement region, in the area of edema, and even in normal appearing brain adjacent to the region of T2 MRI abnormality. (72). It is believed that glioma tumor spreads along the white matter tracts. However Byrne et al showed that MRI failed to demonstrate the disease activity along white matter fiber tracts (73) and also showed that even on enhancing areas of tumor on MRI had extensions of disease (74)

Mosskin et al evaluated clinical utility of MET-PET in glioma imaging. He demonstrated that PET delineates the brain tumor better than CT/MRI but PET was unable to define the tumor grade or could not differentiate active regions from regions with necrosis. (75)

Marks et al demonstrated that the severity of late effects of brain irradiation such as neurocognitive damage is directly proportional to the volume of brain irradiated (76) . A uniform margin on GTV as defined on MRI is expected to cover either too much of normal brain or to could miss certain areas of tumor. The former causes more neurotoxicity and the latter is undesirable as it would result in increased local recurrence due to geographical miss. Therefore margins tailored to tumor extension rather than uniform could improve quality of life as well as local control.

It has been shown in many studies that a dose of 60Gy is necessary for good local control, but this might not be feasible for all patients in view of close proximity to critical organs. Techniques such as MRS could delineate high risk areas within the tumor and this would help to increase the dose with acceptable dose to the critical structures.

Ling et at described the concept of IMRT with dose painting in gliomas which allows customized dose delivery to various parts of the treatment volume based on their dose requirements. He also described the concept of integrating physical and biological conformality in multidimensional conformal radiotherapy (MD-CRT). They emphasized the need for biological images that reveal metabolic, functional, and physiological characteristics of gliomas. (77) and is suitable to use MRS based planning for IMRT (78)

Payne et al showed that with advent of new conformal techniques it is possible to accurately delineate the extent of tumor and also identify regions of high clonogenic density that could be targeted with boost doses of radiotherapy using IMRT. Conventional imaging with CT and MRI has positive predictive value of 50%.

McKnight et al utilized MRS for defining the exact tumor extensions beyond that defined by MRI. The study showed that High Grade Glioma on imaging is a continuous spectrum of disease progression and the tight compartmental differentiation between low grade and high grade areas was not possible on routine imaging and required higher imaging modalities like MRS. They also stated that Choline NAA index (CNI) can be used to help identify metabolically active tumor within and beyond the contrast enhancing lesion that appears on MRI. Wide range of CNI that exist throughout the lesion shows various grades of disease that exist in the lesion and it guides as a tool to target the region with highest histological grade. These features have been shown to help in utilizing CNI as an in vivo parameter for directing biopsies and focal conformal radiotherapy in non-enhancing areas of gliomas.

McKnight et al defined an Abnormality Index (AI) of choline NAA index and defined that all the areas with CNI of more than 2 was the lowest value that showed disease activity in 95% of the patient with HGG.(63) In view of this Pirzkall et al defined and evaluated specifically AI levels of 2, 3, and 4. (64)

Narayana et al utilised MRS in radiotherapy treatment planning and found that post contrast T1W images overestimated the GTV as defined by Choline-Creatine ratio (CCI) of 3 by 40% and the T2W imaging overestimated CTV as defined by CCI of 1 by 30 % indicating over treatment of normal brain tissue.(65)

Volume delineation with MRS in various studies was done taking different metabolite ratios into consideration. Few studies (Narayana et al) considered the ratio of Choline to Creatine into consideration and all those areas with CCI of >/= 3 was taken into a high risk area and those with lesser values of 2 and 1 were defined as intermediate and low risk volumes respectively. Others (Prizkall et al) took Choline to NAA ratio of 4 as high risk area and other areas with lesser CNI of 3 and 2 as intermediate and low risk areas respectively and all areas with CNI of less than or equal to 1 as normal.

Our study was conducted to assess changes in the treatment volume by addition of Magnetic resonance spectroscopy to conventional CT-MRI based volume delineation

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Volume definition:

GTV based on T1 MRI :

Four patients had high risk area on the MRS as shown by CNI ratio of more than 3. When comparison was made between the GTV defined by contrast enhancing lesion defined on T1 MRI and the GTV defined by MRS >3 in these four patients, it was found that MRI defined GTV was more by an average of 37%, the range being 18%-61%. Among the remaining 6 patients, there was no MRS defined high risk area which means that the T1MRI defined GTV was more by 100%.

When the GTV defined by T1 MRI and MRS (CNI 1.5- 3) was compared it was found that MRS defined GTV volume extended beyond MRI defined volume by an average of 32.22 cc and the range was between 3 cc and 111 cc.

Comparison of GTV defined by T1 MR and MRS CNI1.5-3 showed that T1 MR defined GTV was beyond the MRS volume by an average of 13 cc with minimum of 1 cc and maximum of 84.5 cc.

This shows that when T1 MRI is used for GTV definition and when compared with MRS defined GTV, an average volume of 32.22 cc of volume of active disease beyond that defined by MRI alone is seen which if not accounted for can lead to local recurrence.

Volume of GTV defined by T1 MRI alone that is beyond the MRS defined GTV is in an average of 84.5 cc which means that this volume of normal brain is exposed to radiation leading to adverse neurological sequelae.

Narayana et al showed that MRS defined GTV volume was smaller by 40% compared to post contrast T1W imaging defined GTV volumes and in our study it was found to be smaller by 37 %

CTV based on T2 MRI :

Pirzkall et al showed that when MRSI CNI2 was used to define CTV defined by T2MRI the volume was more by 15% .When CNI2alone was used then the volume of CTV was low by 20% compared to T2 alone in grade III tumors and by 40% in grade IV tumors.

Narayana et al noted that T2 based imaging overestimated the CTV defined by MRS by more than 40% indicating overtreatment of normal brain tissue.

In our study, we found that addition of MRS data (CNI 1.5-3)to T2 GTV decreased the average target volume in eight patients by 40%. In the remaining two patients it was increased by 27% and 2% respectively.

PTV based on MRI AND MRS:

Narayana et al showed that MRI FLAIR defined PTV overestimated MRS defined PTV indicating the overtreatment of normal brain tissue. The difference in volumes was however not quantified in his study.

In our study, we found that PTV delineated with MRS data was less by 57 % when compared to PTV delineated with MRI data. This would lead to decreased amount of normal tissue treated , decrease the late radiation effects and improve the quality of life.

Disjoint volume comparison

Pirzkall et al showed that when MRSI CNI2 was used to define CTV defined by T2MRI the volume was more by 15% .When CNI2 alone was used then the volume of CTV was low by 20% compared to T2 alone in grade III tumors and by 40% in grade IV tumors.

Comparison of GTV defined by T2 MR and MRS CNI 1.5-3 showed that T2 MR defined GTV was beyond the MRS volume by an average of 61.58 cc with minimum of 1.3 cc and maximum of 189 cc.

Comparison of GTV defined by T2 MR and MRS CNI 1.5-3 showed that CNI 1.5-3 defined MRS volume was beyond the T2 MRI volume by an average of 7.32 cc with minimum of 1 cc and maximum of 35.8 cc.

Similarly when T2W FLAIR was used alone in defining GTV, volume of GTV defined by MRI that was beyond MRI was about 61.58 cc which indicated the amount of normal brain in the irradiated volume. MRS defined GTV was about 7.32 cc beyond the T2W FLAIR volume which is the active disease not addressed by MRI alone.

CONCLUSIONS

1. PTV defined by MRS was 57% less than that of PTV MRI. This could result in reduction in normal tissue irradiation leading to decreased NT toxicity and facilitate delivery of required doses to the target.
2. T2W MRI defined GTV was more by 40% on an average from MRS (intermediate risk area) defined and the range from 12% -76%. It is possible that a significant amount of normal tissue being irradiated in target volume defined by MRI
3. When T2W FLAIR is used in defining GTV, volume of GTV defined by MRI that was beyond MRS was about 61.58 cc which indicated the amount of normal brain in the irradiated volume.
4. MRS defined GTV was about 7.32 cc beyond the T2W FLAIR volume which is the active disease not addressed by MRI alone.
5. Though there is disjoint volumes, when a margin of 2.5 cm is given on MRI this disjoint volume gets covered in the PTV

Limitations of Magnetic Resonance Spectroscopy:

1) Although MRS is a powerful tool to assess metabolic activity of the gliomas, it has few limitations. Poor quality spectroscopy due to presence of clips, clotted blood and bone within the volume can distort the findings.

2) The normal voxel size used for MRS is 1.5 cm. However for better spatial resolution of the MRS data the size of the voxel will need to be reduced to 1cm and this would lead to increase in scan time (about 40 minutes additional time).

3) The additional duration of Multivoxel MRS Study might hinder its usage in a busy setting and would be difficult to perform the same in poor performance patients

4) Volume delineation by MRS is time consuming and needs a very meticulous evaluation of voxel to voxel for all the metabolic parameters to assess the extent of disease.

5). Currently there is no dedicated software to fuse MRS data via gray scale to MRI for incorporation for routine co registration process with CT scan.

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APPENDIX

Individual Patient wise Characteristics and features of MRI and MRS defined volumes

Patient 1:

Volume of GTV defined by T1MRI – 53.6 cc
Volume of GTV defined by T2 MRI – 253.6 cc
Volume of GTV defined by MRS (CNI >3) – 20.5 cc
Volume of GTV defined by MRS (CNI 1.5-3) – 147.8 cc
Volume of PTV defined by MRI – 1029 cc
Volume of PTV defined by MRS – 534.1 cc

GTV defined by T1 and T2 MRI was larger than the corresponding high and intermediate risk area on MRS. The volume of low risk area and hence the PTV defined by MRS was much smaller than the corresponding volume defined by MRI. The difference of PTV volumes was about 92%.

The PTV volume with MRI was extending into the orbit while the PTV defined by MRS enables sparing of these organs at risk.

Patient 2:

Volume of GTV defined by T1MRI – 17.6 cc
Volume of GTV defined by T2 MRI – 120.6 cc
Volume of GTV defined by MRS (CNI >3) – 14.4 cc
Volume of GTV defined by MRS (CNI 1.5-3) – 34.1 cc
Volume of PTV defined by MRI – 730.2 cc
Volume of PTV defined by MRS – 264.2

GTV defined by T1and T2 MRI were found to be much larger than the corresponding high risk and intermediate risk area on MRS. The volume of PTV in MRI and MRS had large differences and this was about 63%.

Patient 3:

Volume of GTV defined by T1MRI – 90.2 cc
Volume of GTV defined by T2 MRI – 241.4 cc
Volume of GTV defined by MRS (CNI >3) -52.9 cc
Volume of GTV defined by MRS (CNI 1.5-3) – 173.3 cc
Volume of PTV defined by MRI - 1191 cc
Volume of PTV defined by MRS – 532.5 cc

The GTV defined by T1 and T2 MRI was large. The areas with high risk and low risk activity were also found to be high. The PTV was however overestimated by MRI and there was significant difference between the two volumes and this was about 60 %

Patient 4:

Volume of GTV defined by T1MRI – 85.5 cc
Volume of GTV defined by T2 MRI – 173.1 cc
Volume of GTV defined by MRS (CNI >3) – no
Volume of GTV defined by MRS (CNI 1.5-3) – 88.8 cc
Volume of PTV defined by MRI – 821.6 cc
Volume of PTV defined by MRS – 417.3 cc

The GTV defined by T1MRI was found to have intermediate risk activity and hence the GTV defined by T2W FLAIR was found to be over estimating the disease. The low risk area on MRS was much less and there was a large difference in PTV defined by MRI and MRS. This was about 82 %.

Patient 5:

Volume of GTV defined by T1MRI – 31.4 cc
Volume of GTV defined by T2 MRI – 89.8 cc
Volume of GTV defined by MRS (CNI >3) - no
Volume of GTV defined by MRS (CNI 1.5-3) – 53.2 cc
Volume of PTV defined by MRI – 642.8 cc
Volume of PTV defined by MRS – 250.9 cc

GTV defined by T1 MRI was not found to have high risk activity and the intermediate risk area on MRS was found to be much less than the T2 FLAIR defined GTV. Volume of low risk area was small and hence then difference in volumes of PTV defined by MRI and MRS was quite large and this was found to be 60%.

Patient 6:

Volume of GTV defined by T1MRI – 6.1
Volume of GTV defined by T2 MRI – 12.6
Volume of GTV defined by MRS (CNI >3) – no
Volume of GTV defined by MRS (CNI 1.5-3) – 12.9 cc
Volume of PTV defined by MRI – 245.8 cc
Volume of PTV defined by MRS – 109.6 cc

Volume defined by TI GTV, T2W FLAIR was small and there was no high risk area within
T1MRI defined volume. GTV defined by MRS intermediate risk area was more than the GTV defined by the GTV defined by the T2 MRI. The volume of low risk area was large and hence the difference of PTV was 55%.

Patient 7:

Volume of GTV defined by T1MRI – 144.5 cc
Volume of GTV defined by T2 MRI – 175.2
Volume of GTV defined by MRS (CNI >3) no
Volume of GTV defined by MRS (CNI 1.5-3) - 15.4 cc
Volume of PTV defined by MRI - 729.7cc
Volume of PTV defined by MRS – 432 cc

GTV defined by T1 MRI was large but was not found to have higher metabolic activity. Although the enhancing edematous component on T2 FLAIR was large, the volume of intermediate risk area was also found to be low when compared to T2 MRI volume. However the low risk metabolic area was found to be extending beyond and the difference in PTV was about 40 %.

Patient 8:

Volume of GTV defined by T1MRI – 66.5 cc
Volume of GTV defined by T2 MRI – 251.7 cc
Volume of GTV defined by MRS (CNI >3) - no
Volume of GTV defined by MRS (CNI 1.5-3) - 60 cc
Volume of PTV defined by MRI - 771.7 cc
Volume of PTV defined by MRS – 417.8 cc

Although GTV volume defined by T1 MRI was large (66.5 cc) there were no areas with high metabolic activity within this volume. The volume of intermediate risk activity was found to be encompassed within the T2 defined GTV implying that the enhancing edematous component seen on T2 GTV had only an intermediate metabolic activity. The low risk area was however found to be extending much beyond and the difference in PTV between MRI and MRS defined areas was 45%.

Patient 9:

Volume of GTV defined by T1MRI – 5.1 cc
Volume of GTV defined by T2 MRI – 10 cc
Volume of GTV defined by MRS (CNI >3) – no
Volume of GTV defined by MRS (CNI 1.5-3) – 8.4 cc
Volume of PTV defined by MRI – 608 cc
Volume of PTV defined by MRS – 332.4 cc

There were no areas with higher metabolic activity in MRS as shown by absence of areas with CNI >3 and the volume of intermediate risk area were less compared to MRI. The areas with low risk were however seen to be more and the difference in volumes of PTV by MRI and MRS was 45%.

Patient 10:

Volume of GTV defined by T1MRI – 28.9 cc
Volume of GTV defined by T2 MRI -65.1 cc
Volume of GTV defined by MRS (CNI >3) 20.1 cc
Volume of GTV defined by MRS (CNI 1.5-3) 82.9 cc
Volume of PTV defined by MRI – 608 cc
Volume of PTV defined by MRS – 332.4 cc

Volume of GTV defined by MRS with CNI of > 3 was found to be more than that defined by T1 W post gadolinium series. This was found to be especially more at the brain stem area where there was no enhancing component seen but the metabolic activity in terms of areas with CNI >3 and CNI 1.5-3 was found to be more.

This showed that there was difference in location of GTV defined by MRI and MRS and the active disease was found to extend beyond the areas with enhancement.

The PTV defined by T2 MRI + 2.5 cm margin was however found to be encompasing the PTV defined by MRS – showing that the volume of normal brain tissue irradiated was more with MRI defined volumes compared to that by MRS.

This shows that MRS defined volumes were located in differently and was much smaller than the MRI defined volume. The former will help in avoiding tumor recurrence at those areas with high metabolic activity but that was no contrast enhancing and the latter depicts that volume of normal brain tissue irradiated was more causing higher chances of damage to normal brain.

~~81.~~

~~82.~~
~~83.~~

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