

PREDICTING EPILEPTOGENIC POTENTIAL OF BRAIN METASTASES BY GLUTAMATE ANALYSIS USING MAGNETIC RESONANCE SPECTROSCOPY: A RETROSPECTIVE STUDY

Dev Ravishankar¹ and Devika Sunil^{2,*}

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Abstract

Background: Seizures are a common clinical manifestation of a primary or secondary brain tumor and epileptogenic brain secondaries from lung primaries cause significant morbidity around the world. Modern neuroimaging has revolutionized detailed anatomical evaluation of brain tumors but available literature is still scanty on prognosticating epileptogenic potential of brain lesions which if made available will be highly beneficial in making informed clinical decisions. This study is a step towards that endeavor. We in this study test the correlation between Glutamate (Glu) levels in brain secondaries from lung primaries measured non-invasively with MRS as a measure of its epileptogenic potential. **Methods:** We retrospectively over a two year period reviewed clinical and imaging data of 150 patients with brain secondaries from lung primary at our institutions who underwent MRI brain either as part of work-up, seizure protocol or follow up. Brain tumoral Glutamate levels in epileptic and non-epileptic patients were analysed using MRS and its correlation with seizure potential was statistically analyzed using Fisher's exact test to estimate overall effect. A $p \leq 0.05$ was considered statistically significant. **Results:** Of 150 included patients, 57(38%) had seizures, out of which 49(85.6%, $p < 0.005$) showed increase in their brain tumoral Glutamate peak amplitude on MRS which is a marker of increased concentration of this key neuroexcitatory neurotransmitter, thereby suggesting a direct and statistically significant linear correlation between intratumoral Glutamate levels and its epileptogenic potential. **Conclusion:** The findings of this study sheds light on the complex relationship between Glutamate levels within brain secondaries and its epileptogenic potential thereby advancing our understanding of the neurological implications of these lesions and projects MRS as a non-invasive tool in identifying the high risk group for targeted therapeutic interventions including prophylactic antiepileptic therapy.

Keywords: Metabolites; MRS; Neuroexcitatory; Secondaries; Seizure

¹ Department of Radiodiagnosis, Sree Uthradom Thirunal Academy of Medical Science (SUTAMS), Trivandrum, India.
E-mail: devravis@gmail.com

² Department of Radiation Oncology, Sree Gokulam Medical College & Research Foundation (SGMCRF), Trivandrum, India.
E-mail: devikadev91011@gmail.com*

* Corresponding author

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Introduction

Brain metastases particularly from lung primary are common (40-50%) with about 10% of newly detected cases having them at presentation (Barnholtz-Sloan *et al.*, 2004; Avila and Graber, 2010) thereby representing a significant clinical challenge, often leading to neurological complications, including seizures, which can significantly impact the quality of life of affected patients. The epileptogenic potential of these metastatic lesions is complex, involving various pathological processes, including alterations in neurotransmitter levels. In this article, we explore the role of Glutamate (Glu), a key excitatory neurotransmitter, in the epileptogenic potential of brain metastases from lung primary and the potential of Magnetic Resonance Spectroscopy (MRS) in analyzing its content in brain metastatic lesions. Glutamate being the primary excitatory neurotransmitter in the central nervous system and plays a critical role in neuronal signaling, synaptic transmission, and plasticity. While glutamate is essential for normal brain function, excessive glutamatergic activity has been implicated in the pathophysiology of epilepsy (Barker-Haliski and White, 2015). Excitotoxicity, a process in which excessive glutamate leads to neuronal damage and death, is a well-recognized mechanism in epileptogenesis. In the context of brain metastases from lung primary, the dysregulation of glutamate signaling within the tumor microenvironment and surrounding brain tissue can contribute to epileptogenesis. Metabolic changes, disruption of the blood-brain barrier, and altered neuronal excitability associated with these metastatic lesions can lead to aberrant glutamate release and neurotransmission, thereby lowering the seizure threshold (Wong, 2012).

Study by Sarlo (Sarlo and Holton, 2021) using microdialysis and MRS techniques stated that Glutamate metabolites are elevated in epilepsy patients. Analyzing Glutamate content using Magnetic Resonance Spectroscopy (MRS) is a non-invasive imaging method for the *in vivo* detection and quantification of various metabolites, including glutamate, within the brain. Through the application of MRS, it is possible to analyze the glutamate content in brain metastatic lesions from lung primary, providing valuable insights into the biochemical alterations associated with epileptogenesis. MRS utilizes the magnetic properties of certain atomic nuclei, such as hydrogen protons, to generate spectra reflecting the chemical composition of tissues. When placed in a strong magnetic field and subjected to

radiofrequency pulses, these nuclei emit characteristic signals that can be detected and used to generate spectra reflecting the chemical composition of the tissue. By employing specialized MRS sequences and data processing techniques, clinicians and researchers can specifically isolate and quantify the glutamate signal, thereby obtaining valuable information about its concentration within the metastatic lesion and thereby its epileptogenic potential.

The goals of this study were to investigate test-retest reliability for obtaining high-quality spectra, and to quantify the glutamate concentration in brain metastases (Br M) using MRS employing a clinical 1.5T MRI scanner.

Materials and Methods

We retrospectively collected clinical and imaging data of 150 patients during the two year period of June 2021 to June 2023 having brain metastases with lung as primary having undergone MRI brain in our department either for screening, follow up or for seizure evaluation.

Inclusion criteria

Patients with histopathological diagnosis of carcinoma lung with brain metastases in age group of 18 to 80 years old were selected.

Exclusion criteria

Patients with inherent contraindications to undergo MRI like non-MR compatible implants, claustrophobia, inconclusive histopathology reports and prior non-tumoral cause of seizure disorder and past history of brain surgery were excluded.

This study was reviewed and approved by Institutional Ethics Committee of Sree Uthradom Thirunal Academy of Medical Sciences (IEC-SUTAMS). Prior written and informed consent was obtained from all participants after explaining all study aspects. Data acquisition and post-processing were performed by a qualified MR technologist.

Our study was conducted on a 1.5T Siemens Sempra scanner using TE-averaged Point Resolved Spectroscopy (PRESS) method. Subjects were positioned to lie comfortably in the coil, and asked to remain motionless during the procedure. High-resolution T1-weighted anatomic images of the whole brain were acquired by using a three-dimensional fast gradient-echo pulse sequence: TR = 150ms, TE = 8.9 ms, TI = 725 ms, T2=199ms, FOV = 256 × 256 mm, slice thickness =

1.0 mm, flip angle = 9°, TA = 5 min 12s. Images from three orthogonal planes were revealed by using multiplanar reconstruction for localizing the spectroscopic volumes of interest. A 20×20×20mm MRS voxel was planned in the axial, sagittal and coronal planes of the localizer image. The voxel was centered within the lesions along the midline and rotated appropriately to avoid CSF and lipid signal contamination from the ventricle and skull respectively. MRI spectra of metabolites are shown on x and y axis. The x (horizontal) axis displays the chemical shift of the metabolites in units of ppm. The ppm increases from right to left. The y (vertical) axis demonstrates arbitrary signal amplitude of the metabolites. There is evidence of correlation between Glutamate concentrations and median signal amplitude (normal range- 0.6 ± 0.05) at 2.35 ppm in various studies like the one conducted by Dyke *et al*, (2017) and hence MRS signal amplitude can be taken as a marker of metabolite concentration.

The effect of Glutamate levels and seizure potential was statistically analyzed using Fisher's exact test to estimate overall effect. A $p \leq 0.05$ was considered statistically significant.

The peak amplitude was determined using MRS data acquired using specific sequences that target the resonance frequency of glutamate. Spectra are obtained from the region of interest (ROI) localised within the brain metastases using a predefined voxel size and acquisition parameters.

Table 1. Shows Statistical Analysis of study results using Fisher's-exact test

	Elevated Glutamate peak amplitude in range of 0.8 ± 0.05	Normal Glutamate peak amplitude of 0.6 ± 0.05	p-value
Epileptogenic lesions (n=57)	49(85.6%)	8(14%)	$p < 0.005$
Non-epileptogenic lesions (n=93)	4(0.04%)	89(95.6%)	$p < 0.001$

Fisher's-exact test: p -value < 0.05 is statistically significant

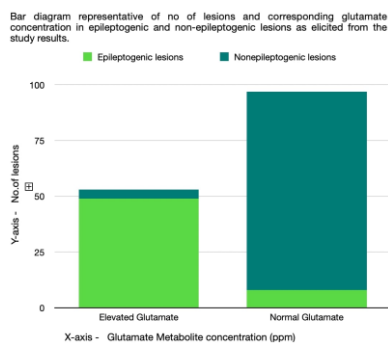


Figure 1. shows Statistical Analysis of study results using Fisher's-exact test

Spectral Processing done using Frequency Domain Analysis wherein the acquired data is processed through Fourier transformation to convert time domain signals into frequency domain spectra.

Baseline Correction was applied to remove any artifacts or baseline drifts that may affect the spectral quality and the peak corresponding to glutamate at around 2.35 ppm was identified within the spectrum.

Peak Amplitude measurement was done with integration, here the area under the peak corresponding to glutamate was calculated by integrating the spectral data around the 2.35 ppm resonance frequency.

For localization of ROI in Epileptogenic Metastatic Lesions in the MRS study and to ensure their consistency across subjects thereby achieve low intra and inter-observer variabilities the following steps were taken which is as follows:

1. Coordinates and Orientation: Coordinates for the ROI can be determined using anatomical landmarks and imaging sequences to identify the lesion location within the brain. The orientation of the ROI can be aligned with the anatomical axes to standardize measurements.
2. Size of ROI: The size of the ROI should encompass the entire metastatic lesion to capture accurate metabolite concentrations. Careful consideration of lesion boundaries and surrounding tissues is essential.
3. Consistency Across Subjects: To ensure consistency across study subjects, using standardized imaging protocols, including acquisition parameters and positioning of the ROI, is crucial. Additionally, inter-observer reliability can be improved by training operators and implementing quality control measures.
4. Reducing Intra and Interobserver Variability: Techniques such as automated ROI placement algorithms and strict adherence to imaging guidelines enabled minimizing intra and interobserver variability. Regular calibration and validation of MRS equipment are also essential.

Results and Discussion

Our study conducted on a 1.5T MRI scanner using TE-averaged MRS PRESS technique obtained a non-overlapping spectrum of glutamate at 2.35 p.p.m which enabled us to compare the glutamate levels in epileptogenic and non-epileptogenic lesions. Of the 150 histopathology proven cases of lung cancer with brain secondaries in our study, 57(38%) had epileptic seizures, out of which 49 (85.6%, $p < 0.005$) (Table 1, Figure1) showed

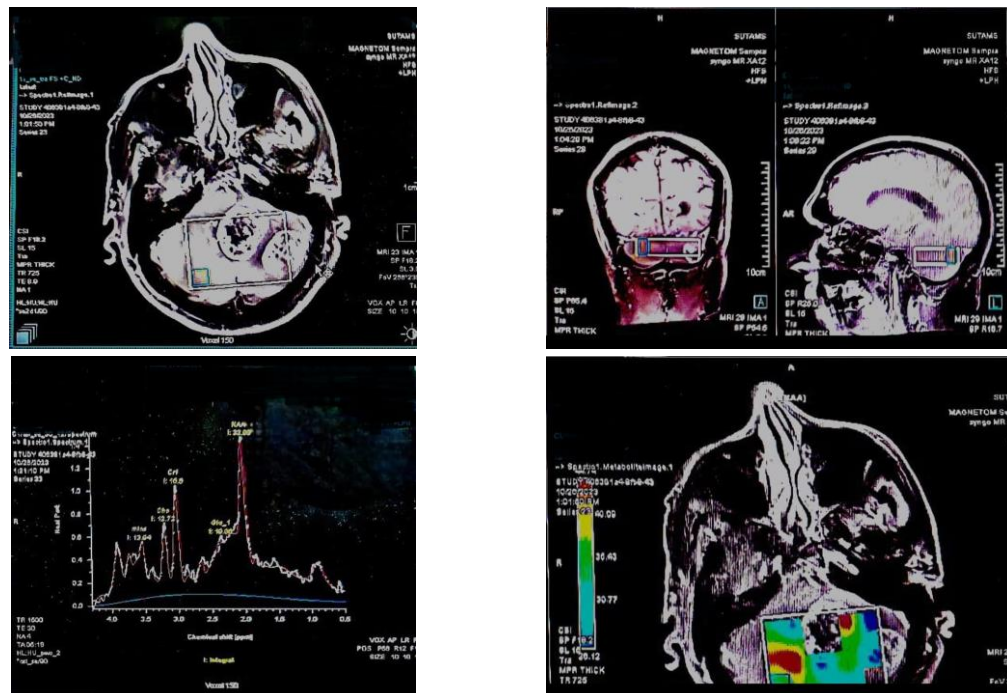


Figure 2. Localized MRS images and MRS spectra of an epileptogenic metastatic lesion. The peak amplitude in nV (y-axis) of Glutamate is 0.8 nV at 2.35 ppm (x-axis, metabolite concentration) which is at a much elevated level thereby suggesting an increased metabolite concentration of Glutamate levels in these lesions.



Figure 3. Lung primary of same patient as in Figure 2a depicted on MRI Thorax (T2 fat-suppressed sequence) of the same patient.

increase in their brain tumoral Glutamate peak amplitude on MRS (Figure 2 and 3) which is a marker of increased concentration of this key neuroexcitatory neurotransmitter, thereby suggesting a linear and statistically significant correlation between Glutamate levels and epileptogenic potential. This was further established by the statistically significant finding in our study that only 4 (0.04%, $p < 0.001$) of the brain secondaries in non-seizure group showed an elevated Glutamate peak (Figure 4) while 89

(95.6%, $p < 0.001$) of them showed a normal Glutamate peak, thereby establishing beyond any reasonable doubt that Glutamate concentration is elevated in epileptogenic brain metastases and that its increased presence in these lesions directly correlates with increase in its epileptogenic potential.

In our study elevated peak amplitude of glutamate peak on MRS was seen in the range of 0.8 ± 0.05 nV as demonstrated by 49 (85.6%) of lesions in seizure group suggesting strong direct correlation while the expected normal peak amplitude range of 0.6 ± 0.05 nV was demonstrated by 89 (95.6%) of the brain tumors in non-seizure group. Importantly only 4 (0.04%) out of 93 brain metastases in non-seizure group showed Glutamate peak in the elevated range of 0.8 ± 0.05 nV while only 8 (14%) of the 57 seizure group brain lesions showed Glutamate peak in the normal range of 0.6 ± 0.05 nV on MRS, while available literature used 3T or higher ultra high field MRI scanner which precludes wider accessibility.

The MRS analysis of glutamate levels within brain metastases from lung primary tumors in our study may have yielded several important findings. Available literature on the topic of MRS in brain tumors is non-specific and limited and most of them are about settled topics like anatomic location, size

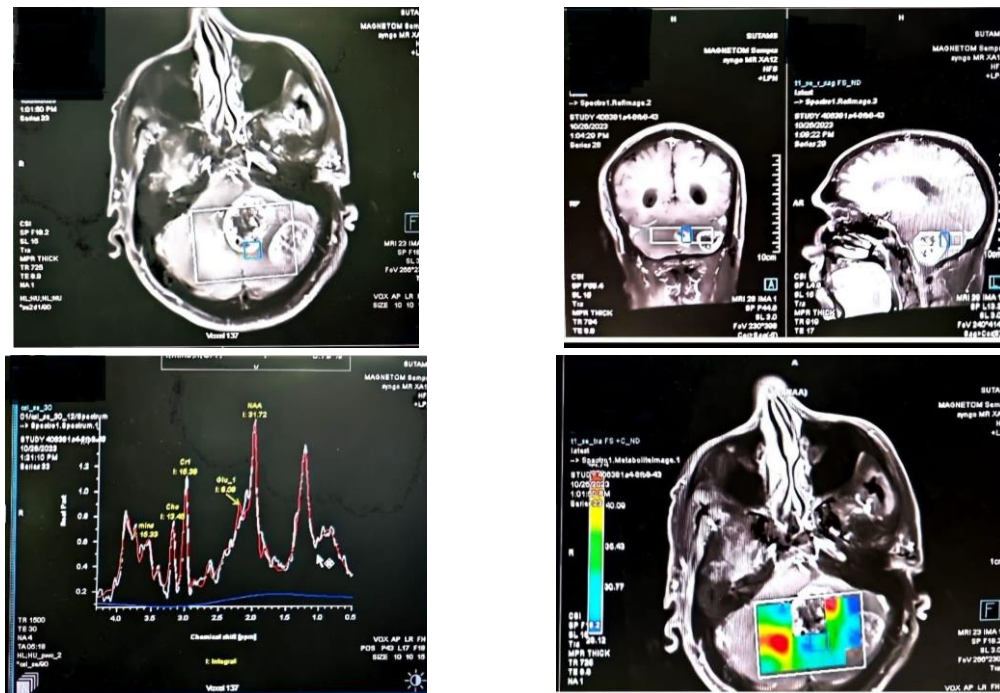


Figure 4. Localized MRS images and MRS spectra of a non- epileptogenic metastatic lesion. The peak amplitude in nV (y-axis) of Glutamate at 2.35 ppm (x-axis, metabolite concentration) is at 0.6nV which is within expected normal range of Glutamate levels in brain tissue.

etc and its role in determining epileptogenic potential of brain tumors like in study by Akeret *et al*, (2019) but is very scarce about brain metastases and their seizure causing potential. As far as we know study has yet been conducted on our topic of research ie MRS analysis of glutamate concentrations in brain metastases and its significance in identifying epileptogenic potential of these tumors. Some studies like the one conducted by Çavuş *et al*, (2016) reported elevations in glutamate concentrations in epileptogenic regions of the brain but these studies were not conducted on brain metastases nor was they measured with MRS but rather with microdialysis. Our study concurred in part with the study by Sarlo and Holton, (2021) that found Glutamate metabolites are elevated in epilepsy patients and epileptogenic regions and they used microdialysis and MRS techniques but their study was not on brain metastases, which was the case in our study.

The markedly increased seizure prevalence (93%) in patients with increased glutamate levels within brain metastases in our study suggests the linear correlation between glutamate levels and seizure potential. In comparison, only 0.04% of non epileptogenic metastases showed increased glutamate peak on MRS thereby providing a comparative analysis of glutamate concentrations in the two subsets. This comparison could offer

insights into the specific role of metastatic lesions in altering local glutamate metabolism and its potential impact on seizure generation. Ours is a pioneering work in this area and to our knowledge no literature is available in this topic for comparison.

In addition to cross-sectional analysis, our study enables identifying the temporal changes in glutamate levels within brain metastases over time, providing insights into the dynamic nature of glutamate metabolism and its potential impact on seizure development and progression. Glutamate analysis using MRS can aid in the differential diagnosis of seizure etiology in patients with brain metastases. Distinguishing between metastasis-related seizures and non-metastasis-related epileptic activity is crucial for appropriate treatment planning. MRS can provide valuable data to support this differentiation by elucidating the glutamatergic alterations specific to the metastatic lesions.

Our study was conducted on a 1.5T Siemens Sempra scanner using TE-averaged MRS technique to obtain a non-overlapping spectrum of glutamate at 2.35 p.p.m and compare the glutamate levels in epileptogenic and non-epileptogenic lesions while whatever available literature has used 3T or higher ultra field MRI scanner which precludes wider accessibility. Also in some of the earlier studies by Pasanta *et al*, (2023) an overlapping of metabolites was noted at the 2.35 ppm which we were able to

mitigate by using TE-averaging PRESS method in MRS thus enabling an accurate measure of Glutamate peak levels in our study.

The findings of the glutamate analysis on MRS of brain metastases from lung primary tumors could have significant implications for understanding the underlying mechanisms of epileptogenicity associated with these metastatic lesions and could contribute to the development of potential biomarkers for predicting and monitoring seizure activity in patients with brain metastases from lung primary tumors. Additionally, the results could inform future research directions aimed at targeted interventions to modulate glutamate levels within metastatic lesions and potentially mitigate their epileptogenic effects. The findings of glutamate analysis on MRS of brain metastases from a primary lung tumor could reveal crucial insights into the biochemical and neurobiological characteristics of these metastatic lesions in relation to their epileptogenic potential.

MRS being a non-invasive imaging technique that can provide information about the chemical composition of tissues, including in the context of brain metastatic lesions from lung primary, it can be a valuable tool for analyzing not only the levels of glutamate, but other important neurotransmitter in brain thereby shedding light on various other neurological disorders. To our knowledge no in vivo comparisons between different brain metastases from lung primaries have been made. This study therefore aimed to investigate the possibility of an elevated Glutamate peak and therefore increased glutamate concentration in epileptogenic brain metastatic by virtue of being the key excitatory neurotransmitter in brain. Our study statistically proved this hypothesis. Using MRS to analyze glutamate levels in brain metastatic lesions from lung primary can provide important insights into the biochemical changes associated with these lesions. The ability to non-invasively quantify glutamate levels in these lesions can aid in diagnosis, treatment planning and monitoring of treatment response.

Furthermore, MRS can also provide insights into other metabolites and biochemical markers associated with brain metastases, such as choline, N-acetylaspartate (NAA), and lactate. By examining the overall metabolic profile of the lesion, clinicians can gain a better understanding of its biological behavior and potentially identify targets for therapeutic intervention. In the context of lung primary metastatic lesions, MRS in tandem with conventional MRI can also play a crucial role in differential diagnosis by distinguishing between metastatic lesions and other brain pathologies, such as primary brain tumors or inflammatory processes. This can help guide treatment decisions and avoid

unnecessary invasive procedures. Additionally, MRS can be used to monitor the response to treatment in brain metastases. Changes in glutamate levels and other metabolic markers over the course of treatment can provide valuable information about treatment efficacy and help guide clinical management. Overall, the use of MRS for glutamate analysis in brain metastatic lesions from lung primary holds great promise for improving our understanding of the biochemical underpinnings of these lesions and guiding personalized treatment approaches. Further research and clinical studies in this area can help elucidate the clinical utility of MRS in the management of brain metastases and contribute to improved patient outcomes.

The ability to analyze glutamate content in brain metastatic lesions using MRS can have profound clinical implications in the management of patients with lung primary metastases experiencing seizures. By gaining insights into the glutamate levels within these lesions, clinicians can better understand the epileptogenic potential of the metastases and tailor treatment strategies accordingly.

Furthermore, monitoring changes in glutamate levels over the course of treatment can serve as an indicator of treatment response and aid in the optimization of therapeutic interventions. By leveraging MRS to track glutamate dynamics, clinicians can assess the impact of treatments on the epileptogenic potential of brain metastatic lesions and make informed adjustments to patient care.

In the case of glutamate analysis, MRS can specifically target the glutamate signal and provide quantitative information about its concentration in the tissue. This can be achieved by selecting the appropriate spectral acquisition parameters and data processing methods to isolate and quantify the glutamate signal. Interpreting the MRS data involves comparing the glutamate signal with other spectral peaks and using reference standards to determine the absolute concentration of glutamate in the tissue. This quantitative information can then be used to assess the biochemical status of the brain metastatic lesion and its potential impact on neurological function.

Future directions and research opportunities in the field of glutamate analysis and its relationship to the epileptogenic potential of brain metastases from lung primary using MRS holds great promise. Prospective studies investigating the correlation between glutamate levels, seizure occurrence, and treatment outcomes can provide critical insights into the underlying mechanisms of epileptogenesis and guide the development of targeted interventions.

Moreover, the integration of advanced MRS techniques, such as functional MRS and

spectroscopic imaging, can expand our understanding of spatial and functional heterogeneity in glutamate distribution within brain metastatic lesions. To not lose sight of the primary objective of our study which is to have an objective measure of the the epileptogenic potential of brain metastases from lung primary tumors using Glutamate metabolic analysis by MRS, we consciously chose not to consider other settled contributory factors to seizures in brain metastases like increased age at presentation, increased tumor size and cortical based tumor location in temporal/frontal regions which are already commonly available in literature by Lee *et al.*, (2010). The ability to analyze glutamate content in brain metastatic lesions using MRS can have profound clinical implications in the management of patients with lung primary metastases experiencing seizures. By gaining insights into the glutamate levels within these lesions, clinicians can better understand the epileptogenic potential of the metastases and tailor treatment strategies accordingly.

Comparison with other imaging modalities for Glutamate detection Besides MRS, other imaging modalities and methods can be used to determine glutamate levels in metastatic brain lesions:

1. Positron Emission Tomography (PET): PET imaging using specific glutamate tracers can provide quantitative information about glutamate levels in the brain (Kim *et al.*, 2020).
2. Magnetic Resonance Imaging (MRI): Advanced MRI techniques like diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) can indirectly assess glutamate levels based on tissue characteristics (Banihashemi *et al.*, 2023).
3. Microdialysis: Invasive technique that allows for direct measurement of neurotransmitter concentrations, including glutamate, in the brain extracellular space (Chefer *et al.*, 2009).

Viable biomarkers for determining Glutamate Levels in brain metastatic lesions include:

1. Glutamate Receptors: Expression levels of glutamate receptors like N-methyl-D-aspartate (NMDA) receptors can provide insights into glutamate signaling in metastatic lesions (Kim *et al.*, 2020).
2. brain metastases.
3. Cerebrospinal Fluid (CSF) Markers: Glutamate levels in the CSF can be indicative of glutamate dysregulation in brain metastatic lesions (Miladinovic *et al.*, 2015).
4. Glutamate Transporters: Monitoring glutamate

transporter proteins such as excitatory amino acid transporters (EAATs) can indicate glutamate clearance mechanisms in the brain (Magi *et al.*, 2019).

5. Glutamate Metabolism Enzymes: Enzymes involved in glutamate metabolism, such as glutamate decarboxylase and glutaminase, can serve as biomarkers for glutamate levels in brain metastases (Lieu *et al.*, 2020).

Conclusions

In conclusion, the epileptogenic potential of brain metastases from lung primary is multifaceted and involves complex neurobiological processes, including alterations in glutamate signaling. The application of Magnetic Resonance Spectroscopy for the analysis of glutamate content within these metastases offers a novel approach to elucidate the underlying mechanisms of epileptogenesis and personalize the management of affected patients. By leveraging the capabilities of MRS, we can enhance our understanding of the neurochemical underpinnings of epileptogenesis and pave the way for localizing epileptogenic tumor foci and effecting targeted therapeutic approaches, including focal surgical resection or stereotactic radiosurgery in medically refractory seizures.

Future directions and research opportunities in the field of glutamate analysis and its relationship to the epileptogenic potential of brain metastases from lung primary using MRS holds great promise. Prospective studies investigating the correlation between glutamate levels, seizure occurrence, and treatment outcomes can provide critical insights into the underlying mechanisms of epileptogenesis and guide the development of targeted interventions. Moreover, the integration of advanced MRS techniques, such as functional MRS and spectroscopic imaging, can expand our understanding of spatial and functional heterogeneity in glutamate distribution within brain metastatic lesions.

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