Diagnostic value of posttherapy brain single photon emission computed tomography/computed tomography with pentavalent ^{99m}Tc dimercaptosuccinic acid in patients with glioblastoma multiform: preliminary report

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Aim

To evaluate the feasibility of performing ^{99m}Tc dimercaptosuccinic acid (DMSA) (V) brain single photon emission computed tomography/computed tomography (SPECT/CT) in patients with glioblastoma multiform after their definitive therapy.

Patients and methods

Patients with documented grade IV glioma were prospectively recruited for this study. ^{99m}Tc-DMSA (V) brain SPECT/CT imaging was acquired after a mean interval of 76 ± 46 days from therapy, 2–3 h. After intravenous injection of 555–740 mBq of the tracer. Scans were interpreted visually as positive or negative by three nuclear medicine physicians. Agreement between two or more physicians was considered a consensus decision. The consensus results of DMSA (V) SPECT/CT were compared against the reference standard which was based on subsequent clinical/neuroimaging follow up or pathology whenever resurgery is performed. Lesion quantitation was performed by one nuclear medicine physician by drawing a region of interest on the lesion site (L) and a mirror region of interest on the contralateral normal brain tissue (NL) then L/NL ratio was calculated.

Results

A total of 20 patients were enrolled in this study. According to the reference standard, recurrence was detected in 10 patients while 10 were disease free. Interreader kappa agreement ranged from 0.65 to 0.90. Consensus reading of DMSA (V) SPECT/CT correctly detected recurrence in 8/10 (sensitivity 80%) and correctly ruled out disease in 9/10 (specificity 90%). L/NL ratio for positive and negative cases were 6.6 ± 8 and 1.3 ± 1.1, respectively ($P < 0.001$).

Conclusion

^{99m}Tc-DMSA (V) brain SPECT/CT is feasible and may be a specific noninvasive diagnostic tool for the follow-up of patients with glioblastoma multiform after chemoradiotherapy.

Keywords:

^{99m}Tc dimercaptosuccinic acid (V), brain single photon emission computed tomography/computed tomography, glioblastoma multiform

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Introduction

Glioblastoma multiform (GBM) is the most common primary malignant brain neoplasm in adults and carries a poor prognosis. Treatment involves maximum safe excision whenever possible, followed by radiotherapy and concurrent chemotherapy. Nevertheless, nearly all tumors recur after a relatively short period of time with median progression free-survival ranging from 5.5 to 13 months. Moreover, about 90% of recurrences occur within the high-dose radiation field [1].

Radiation therapy to the brain may lead to postradiation injury which typically occurs within the first 3 to 12 months after radiotherapy but it was reported up to several years and even decades later. The clinical picture of radiation necrosis (RN) is variable and may include seizures, headache, personality changes, and neurologic deficits. These symptoms mimic tumor recurrence and differentiation between the two entities clinically or even by conventional radiological modalities is difficult. Pathological diagnosis remains the gold standard for this diagnostic dilemma but it is invasive and poses a risk of misdiagnosis due to heterogeneity of brain lesions with coexisting RN and tumor cells [2].

This discrimination is necessary because the two conditions have different treatment modalities and prognosis [2].

Several noninvasive imaging modalities have been evaluated for the differentiation between RN and residual/recurrent glioma including MRI techniques, single photon emission computed tomography (SPECT), and positron emission tomography (PET), however categorization is still challenging [3].

18F-FDG was the first PET tracer used for imaging brain tumors. However, the high background levels of cortical glucose, and the frequent false positive results from inflammatory process and subclinical seizure activity were reported as confounding [4]. Radiolabeled amino acids might be preferable over FDG due to relatively increased uptake in the tumor compared with normal brain tissue as it directly image the rate of proliferation. However, PET, especially with radiolabeled amino acids, has several disadvantages; being expensive and not widely available, in addition to the short half-life of its tracers [5].

SPECT has the advantages of being more widely available and less expensive. Multiple SPECT tracers have been explored. Of them Thallium-201 and 99 mTc-MIBI are, possibly, the most extensively discussed. The main disadvantage of 201Tl is the lower spatial resolution, relatively large radiation dose, and the reported high false positive results. Normal uptake of MIBI in the choroid plexus may be confounding for assessing tumors around the ventricles [3].

Pentavalent ^{99m}Tc dimercaptosuccinic acid (^{99m}Tc (V) DMSA), is a nonspecific tumor targeting SPECT radiotracer, that has been used for imaging of various tumors including lung and breast carcinoma [6,7]. However, to date, scarce reports discussed the utility of DMSA-V in patients with glioma [3].

The aim of this study was to evaluate the feasibility of performing ^{99m}Tc-DMSA (V) brain SPECT/CT in patients with GBM after their definitive therapy.

Patients and methods

Patients

This prospective study was approved by Institutional Review Board and all patients signed an informed consent. We recruited consecutive patients presenting for follow-up at our oncology clinics. All patients had a pathologically proven grade IV glioma (GBM) or typical imaging features.

We excluded severely ill patients and those with disturbed conscious level, in addition to patients who were judged that they cannot lie down comfortably without movement for at least 20 min. Seventeen patients underwent surgical resection of the primary tumor followed by concurrent radiochemotherapy; the remaining three patients received their definitive therapy based on the typical imaging features of GBM. Patients were referred for SPECT/CT scanning on average 10 weeks after ending their definitive/adjuvant therapy.

Scanning protocol

^{99m}Tc (V) DMSA preparation was performed according to the method described by Hirano *et al.* [8]. In short, 200 μl of 7% sodium bicarbonate solution ($NaHCO_3$) were added to the commercially available DMSA kit containing 1.4 mg DMSA and 0.5 mg $SnCl_2$-$2H_2O$ as a reducing agent, then reconstituted with 2 ml of ^{99m}Tc-pertechnetate solution.

A dose of 555–740 MBq ^{99m}Tc (V) DMSA was injected into a peripheral vein. Delayed brain SPECT/CT images were obtained at 2–3 h after injection.

The patient was asked to lie flat in a supine position with the neck hyperextended and the hands by the side. To ensure no movement, patient's head was strapped on a special brain scanning bed pallet provided by the manufacturer. Imaging was performed on a hybrid dual head SPECT/CT machine (Symbia T, Siemens, Erlangen, Germany) fitted with a low energy high-resolution collimator, using 15% energy window set at 140 keV.

SPECT images were acquired in a noncircular 360° arc for a total of 64 frames, 25-s/frame, using a 128 × 128 matrix size. Following SPECT acquisition, low-dose CT was acquired for anatomical localization and attenuation correction. The used CT parameters were: tube voltage 130 kV, tube current 80 mA, and slice thickness 1 mm.

The total SPECT/CT acquisition time was about 20 min. Images were reconstructed using manufacturer's iterative protocol (four iterations, four subsets, and Gaussian filter 8 mm).

The reconstructed images were reviewed on a viewing workstation running OsiriX MD version 6.5.2 (Pixmeo, Bernex, Switzerland). Each study was displayed as SPECT, CT, and fused images reconstructed in the three standard projections (axial, coronal, and sagittal).

Data reading

Tracer accumulation in the brain was evaluated visually by three nuclear medicine physicians blinded to the clinical data and independent from each other. Readers'

experience was: 10, 22, and 15 years for readers 1, 2, 3, respectively.

Reader 1 was asked to reread all the studies at least 3 months after the first reading. The second reading was considered for the consensus reporting. Also, the same reader quantified the lesions using mirror regions of interest (ROIs) on the lesion and on the contralateral normal brain tissue to calculate the lesion to nonlesion ratio (L/NL ratio) by dividing mean count of the lesion ROI by that of the nonlesion ROI.

For reporting, the methodology described by Henze *et al.* [9] was used; where focally increased uptake, compared with the contralateral side was considered abnormal.

Data were recorded for each reader as either positive or negative. A consensus reading was considered on the basis of agreement between two (or three) of the physicians reporting the studies.

Reference standard

Subsequent neuroimaging follow-up was used as the reference test. Histopathology is considered the gold standard whenever resurgery was performed as part of the patient's standard of care.

Statistical analysis

Qualitative data were expressed as frequencies and percentages; whereas quantitative data were summarized and expressed as mean ± SD and median (range). Agreement within or between different readers was measured using kappa test. The sensitivity, specificity, accuracy values for ^{99m}Tc (V) DMSA were calculated in relation to the reference standard. Differences in uptake ratios between the negative and positive groups was measured using Mann–Whitney test. The 95% confidence interval was reported whenever possible. In all analyses a two-tailed *P* value less than 0.05 were considered significant. Data were analyzed using SPSS software package, version 21.0 software (IBM Corp., Armonk, New York, USA).

Results

Patients

A total of 22 eligible patients were initially recruited for our study, two of them died before adequate follow-up to ensure independent validation of the study findings, leaving a valid cohort of 20 patients for analysis.

Of the 20 patients, nine were females and 11 were males, with a median age of 40 (range, 7–68 years). Only one child was included in the study. Most tumors were located at the parietal lobe (11/20), 4 in the temporoparietal region, two in the frontoparietal region, two in the frontal, and one in the temporal lobes.

Seventeen patients underwent surgery (debulking or excisional biopsy). The average size of the tumor was 4.9 ± 1.4 cm while the average tumor bulk removed during surgery was 3.0 ± 1.1 cm. All the 17 patients were grade 4 gliomas by pathology. In the three that did not undergo surgery, the typical imaging features were enough to make the diagnosis of GBM [10].

All patients continued follow-up for a mean duration of 13 ± 7 months after the SPECT/CT study. According to the final follow-up status, recurrence was confirmed in 10 patients (four females, six males). Of the 20 patients, only four patients underwent biopsy or resurgery (three were proved recurrent and one was having gliosis). The rest of the patients underwent standard of care neuroimaging follow-up using MRI. At the end of the study, nine patients died, six of them with recurrence. Four patients with recurrence were alive.

Single photon emission computed tomography/ computed tomography findings

The three nuclear medicine physicians agreed on the readings of 17 studies. Agreement levels between the three readers is reported in Table 1. Overall, the average agreement between the three readers was 0.8.

By consensus reading, 8/10 positive scans were proved true positive giving a sensitivity of 80% [95% confidence interval (CI): 44–97); 9/10 negative scans were proved true negative (Fig. 1) with a specificity of 90% (95% CI: 56–100). Overall, one false positive and two false negative results were encountered. The positive predictive value was 89% (95% CI: 52–99). The overall accuracy was 85% (95% CI: 62–97).

The lesions that were proved positive showed a L/NL ratio of 6.6 ± 8.1 compared with 1.3 ± 1.1 in the lesions that were proved negative ($P < 0.001$).

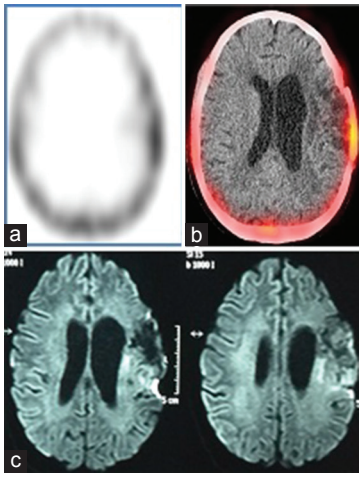
At the end of the study, 5/10 patients with positive scans died (overall survival = 31%) compared with 4/10 with negative SPECT/CT findings (overall survival = 57%). The difference in survival was not statistically significant.

Table 1 Level of agreement between readers

	*R*1	*R*2	*R*3
*R*1	0.80 (0.49-1.0)[a]	0.70 (0.38-1.0)	0.90 (0.69-1.0)
*R*2	-	-	0.80 (0.53-1.0)

[a]Intraobserver agreement reported for *R*1 only.

Figure 1



Male patient, 35-year old with left parietal GBM. (a) SPECT and (b) fused SPECT/CT with ^{99m}Tc (v) DMSA showed no abnormal activity within the brain. (c) MRI showed enhancing lesion suggesting recurrence. Second biopsy 1 year later revealed gliosis. CT, computed tomography; DMSA, dimercaptosuccinic acid; GBM, glioblastoma multiform; SPECT, single photon emission computed tomography.

Also, the L/NL ratios overlapped significantly between the group that died and the group that were alive at the end of follow-up (4.3 ± 8.3 vs. 3.4 ± 2.7; $P = 0.5$).

Discussion

GBM is highly aggressive primary brain tumor. Despite the development of several novel therapeutic targets for chemotherapy and immunotherapy, none of them have proven effective. Progression remains rapid due to aggressive nature of the tumor [11].

Noninvasive assessment of glioma may have an important role in treatment planning, selection of patients for novel therapies, monitoring therapy response, biopsy guidance, and evaluation of prognosis [12]. However, the current tools used for imaging glioma progression may be suboptimal and there is clinical need for inexpensive and widely available imaging techniques.

In this prospective study, we explored the feasibility of performing ^{99m}Tc (V) DMSA SPECT/CT scanning in a group of patients with GBM.

The preparation of (V) DMSA from commercial kits has been well-established and validated in literature. The method is easy, fast, and inexpensive [8].

The mechanism of uptake of (V) DMSA is closely related to the structural similarity between its core and the phosphate (PO_4^{3-}) anion. Inorganic phosphate (Pi) is involved in a large number of biochemical processes and contributes to the structure of DNA, RNA, proteins, and phospholipids. There is a consensus that tumor cells require relatively more phosphate because of their rapid rates of growth [13]. Phosphate enters cells via three different types of energy-dependent sodium/phosphate (NaPi) cotransporters named types I, II, and III. Type I NaPi (also called NPT1) cotransporters are expressed in the liver and kidney. Type II (also called NPT2) has been found in brain, osteoclasts, lung, small intestine, and proximal tubules [14]. Type III NaPi cotransporter (designated PiT1 PiT2 in humans) is widely distributed in mammalian tissue where it functions as housekeeping gateway for intracellular Pi homeostasis. Denoyer *et al.* [15] demonstrated that (V) DMSA uptake is specifically mediated by PiT1 type III transporter.

PiT1 expression is regulated by the EGFR signaling. EGFR tyrosine-kinase inhibitors, including gefitinib, have been included in the management in many cancers and explored in others. In three glioblastoma lines treated with gefitinib, a strong decrease in the Pi transport was noted; secondary to 40% inhibition in PiT1 expression. Also, it was demonstrated that when PiT1 gene is silenced, the transfected cells showed 30% more sensitivity to tumor necrosis factor-induced apoptosis and resulted in 40% reduction in the number of cancer cells after 72 h of transfection [13].

Previous research by Hirano and colleagues demonstrated that regional blood–brain barrier disruption is a prerequisite for (V) DMSA tracer penetration into the vascular bed of a lesion. However, tracer retention inside these cells is maintained by distinct cellular mechanisms acting independently from the loss of barrier integrity [16].

Other reports have shown that (V) DMSA uptake is strongly correlated with cellular proliferation as measured by the Ki-76 index [17].

Taken together, these findings may represent the molecular rationale for using (V) DMSA as a surrogate for imaging cellular proliferation which can possibly be exploited for therapy monitoring.

In this study we recruited patients with GBM after definitive or adjuvant chemoradiotherapy. We demonstrated the feasibility of quantitative and qualitative evaluation of ^{99m}Tc (V) DMSA SPECT/CT scan in these patients. Visual assessment of the images was reproducible among physicians with different levels of experience. The average agreement between three experienced physicians was 80%. On the other hand, the consensus agreement between two (or the three) physicians provided overall accuracy of 85% with high positive predictive value of 89% (95% CI: 52–100).

We encountered three false results (two negative and one positive). In the false positive study (Fig. 2), intense tracer uptake was seen in the surgical wound, which seems continuous with intra-axial focus of tracer activity corresponding to a site of calcification. We assumed that calcification might be responsible for the false positive uptake. ^{99m}Tc (V) DMSA is known to show uptake at sites of active calcification/bone formation [18]. Other investigators showed that low-grade tumors and few cases of RN could have faint to negligible (V) DMSA uptake, congruent with their low proliferation rate [19]. The two false negative ^{99m}Tc (V) DMSA cases may be explained by the small volume of the active tumor mass which is in agreement with previous results reported by Amin *et al.* [3] who attributed the cause of false negative to be the small size of the lesion.

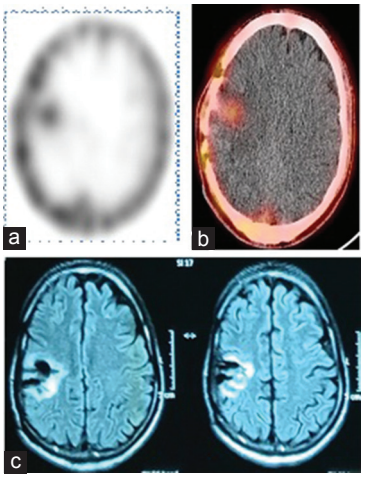
In line with our reported high sensitivity, Amin *et al.* [3] evaluated the diagnostic role of ^{99m}Tc (V) DMSA in 24 patients with a spectrum of all grades of glioma and demonstrated a sensitivity approaching 90% compared with only 61% for single voxel magnetic resonance spectroscopy (1H-MRS) [3]. To ensure uniformity, and to concentrate our effort, we focused this work to only patients with grade IV glioma.

Previous works by Hirano and colleagues, demonstrated the relationship between (V) DMSA retention and histologic grade of the malignancy. They found that gliomas exhibit gradual increase in their tracer uptake on delayed imaging at 2–3 h after injection compared with early imaging. The retention parameters significantly reflected tumor histology; being more with higher grades like GBM and hence they propose that (V) DMSA can differentiate benign from malignant changes [16]. Our quantitative analysis supports this finding. We observed a lesion to nonlesion count ratio of 1.3 ± 1.1 in negative lesions compared with 6.6 ± 8.1 in positive ones. This finding support the use of quantification as an adjunct tool for identifying benign from malignant lesions.

Although our sample size is small, and the patients were recruited at variable time points after finishing their therapies, however, its prospective design, enrollment of only one tumor grade, and homogeneous acquisition and processing protocol in addition to reading by three physicians are all merits for our report. To our knowledge, this would be the first study that utilized SPECT/CT for imaging ^{99m}Tc (V) DMSA in uniform cohort of GBM patients.

In conclusion, ^{99m}Tc (V) DMSA SPECT/CT scanning is feasible and reproducible in patients with GBM after therapy. The reported high specificity of this technique is promising and warrant further exploration of its utility in larger group of patients.

Figure 2



Male patient, 32-year old with GBM in right parietal region, underwent excisional biopsy. (a) SPECT and (b) fused SPECT/CT with ^{99m}Tc (v) DMSA showed abnormal focal activity in the right parietal region. (c) MRI 7 months later showed evidence of gliosis. CT, computed tomography; DMSA, dimercaptosuccinic acid; GBM, glioblastoma multiform; SPECT, single photon emission computed tomography.

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Conflicts of interest

There are no conflicts of interest.

References

1 Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. Asian Pac J Cancer Prev 2017; 18:3.

2 Alexiou GA, Tsiouris S, Kyritsis AP, Voulgaris S, Argyropoulou MI, Fotopoulos AD. Glioma recurrence versus radiation necrosis: accuracy of current imaging modalities. J Neuro-oncol 2009; 95:1–11.

3 Amin A, Moustafa H, Ahmed E, El-Toukhy M. Glioma residual or recurrence versus radiation necrosis: accuracy of pentavalent technetium-99m-dimercaptosuccinic acid [Tc-99m (V) DMSA] brain SPECT compared to proton magnetic resonance spectroscopy (1 H-MRS): initial results. J Neuro-oncol 2012; 106:579–587.

4 Tie J, Gunawardana DH, Rosenthal MA. Differentiation of tumor recurrence from radiation necrosis in high-grade gliomas using 201 Tl-SPECT. J Clin Neurosci 2008; 15:1327–1334.

5 Terakawa Y, Tsuyuguchi N, Iwai Y, Yamanaka K, Higashiyama S, Takami T, *et al.* Diagnostic accuracy of 11C-methionine PET for differentiation of recurrent brain tumors from radiation necrosis after radiotherapy. J Nucl Med 2008; 49:694–699.

6 Hirano T, Otake H, Yoshida I, Endo K. Primary lung cancer SPECT imaging with pentavalent technetium-99m-DMSA. J Nucl Med 1995; 36:202–207.

7 Papantoniou V, Sotiropoulou E, Valsamaki P, Tsaroucha A, Sotiropoulou M, Ptohis N, *et al.* Breast density, scintimammographic 99m Tc (V) DMSA uptake, and calcitonin gene related peptide (CGRP) expression in mixed invasive ductal associated with extensive *in situ* ductal carcinoma (IDC+DCIS) and pure invasive ductal carcinoma (IDC): correlation with estrogen receptor (ER) status, proliferation index Ki-67, and histological grade. Breast Cancer 2011; 18:286–291.

8 Hirano T, Tomiyoshi K, Zhang YJ, Ishida T, Inoue T, Endo K. Preparation

and clinical evaluation of technetium-99m dimercaptosuccinic acid for tumour scintigraphy. Eur J Nucl Med 1994; 21:82–85.

9 Henze M, Mohammed A, Schlemmer HP, Herfarth KK, Hoffner S, Haufe S, *et al*. PET and SPECT for detection of tumor progression in irradiated low-grade astrocytoma: a receiver-operating-characteristic analysis. J Nucl Med 2004; 45:579–586.

10 Asari S, Makabe T, Katayama S, Itoh T, Tsuchida S, Kunishio K, *et al*. Evaluation of MRI score in the differentiation between glioblastoma multiforme and metastatic adenocarcinoma of the brain. Acta Neurochir (Wein) 1993; 122:54–59.

11 Donche S, Verhoeven J, Descamps B, Bolcaen J, Deblaere K, Boterberg T, *et al*. The path toward PET-guided radiation therapy for glioblastoma in laboratory animals: a mini review. Front Med (Lausanne) 2019;6:5.

12 Alexiou GA, Tsiouris S, Kyritsis AP, Fotakopoulos G, Goussia A, Voulgaris S, *et al*. The value of 99mTc-tetrofosmin brain SPECT in predicting survival in patients with glioblastoma multiforme. J Nucl Med 2010; 51:1923–1926.

13 Lacerda-Abreu MA, Russo-Abrahão T, de Queiroz Monteiro R, Rumjanek FD, Meyer-Fernandes JR. Inorganic phosphate transporters in cancer: functions, molecular mechanisms and possible clinical applications. Biochim Biophys Acta Rev Cancer 2018; 1870:291–298.

14 Werner A, Dehmelt L, Nalbant P. Na+-dependent phosphate cotransporters: the NaPi protein families. J Exp Biol 1998; 201:3135–3142.

15 Denoyer D, Perek N, Le Jeune N, Frere D, Dubois F. Evidence that 99m Tc-(V)-DMSA uptake is mediated by NaPi cotransporter type III in tumour cell lines. Eur J Nucl Med Mol Imag 2004; 31:77–84.

16 Hirano T, Otake H, Shibasaki T, Tamura M, Endo K. Differentiating histologic malignancy of primary brain tumors: pentavalent technetium-99m-DMSA. J Nucl Med 1997; 38:20–25.

17 Papantoniou VJ, Souvatzoglou MA, Valotassiou VJ, Louvrou AN, Ambela C, Koutsikos J, *et al*. Relationship of cell proliferation (Ki-67) to 99m Tc-(V) DMSA uptake in breast cancer. Breast Cancer Res 2004; 6:R56.

18 Lam AS, Puncher MR, Blower PJ. *In vitro* and *in vivo* studies with pentavalent technetium-99m dimercaptosuccinic acid. Eur J Nucl Med 1996; 23:1575–1582.

19 Tsiouris S, Pirmettis I, Chatzipanagiotou T, Ptohis N, Papantoniou V. Pentavalent technetium-99m dimercaptosuccinic acid [99m Tc-(V) DMSA] brain scintitomography – a plausible non-invasive depicter of glioblastoma proliferation and therapy response. J Neurooncol 2007; 85:291–295.