

Treatment Of Glioblastoma Multiforme With “classic” 4:1 Ketogenic Diet Total Meal Replacement

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Abstract

Background: Glioblastoma (GBM) has poor survival with standard treatment. Experimental data suggest potential for metabolic treatment with low carbohydrate ketogenic diet (KD). Few human studies of KD in GBM have been done, handicapped by difficulty and variability of the diet, compliance and feasibility issues. We have developed a novel KD approach of total meal replacement (TMR) program using standardized recipes with ready-made meals. This pilot study evaluated feasibility, safety, tolerability and efficacy of GBM treatment using TMR program with “classic” 4:1 KD.

Methods: GBM patients were treated in an open-label study for 6 months with 4:1 [fat]:[protein + carbohydrate] ratio by weight, 10 g CH/day, 1600 kcal/day TMR. Patients were either newly diagnosed (group 1) and treated adjunctively to radiation and temozolomide, or had recurrent GBM (group 2). Patients checked blood glucose and blood and urine ketone levels twice daily and had regular MRIs. Primary outcome measures included retention, treatment-emergent adverse events (TEAEs) and TEAE-related discontinuation. Secondary outcome measures were survival time from treatment initiation and time to MRI progression.

Results: Recruitment was slow, resulting in early termination of the study. Eight patients participated, 4 in group 1 and 4 in group 2. Five (62.5%) subjects completed the 6 months of treatment, 4/4 subjects in group 1 and 1/4 in group 2. Three subjects stopped KD early: 2 (25%) because of GBM progression and one (12.5%) because of diet restrictiveness. Four subjects, all group 1, continued KD on their own, three until shortly before death, for total of 26, 19.3 and 7 months, one ongoing. The diet was well tolerated. TEAEs, all mild and transient, included weight loss and hunger (n=6) which resolved with caloric increase, nausea (n=2), dizziness (n=2), fatigue and constipation (n=1 each). No one discontinued KD because of TEAEs. Seven patients died. For these, mean (range) survival time from diet initiation was 20 months for group 1 (range 9.5-27) and 12.8 months for group 2 (6.3-19.9). Mean survival time from diagnosis was 21.8 months for Group 1 (11-29.2) and 25.4 for group 2 (range 13.9-38.7). One patient with recurrent GBM and progression on bevacizumab experienced a remarkable symptom reversal, tumor shrinkage and edema resolution 6-8 weeks after KD initiation and survival for 20 months after starting KD.

Conclusions: Treatment of GBM patients with 4:1 KD using total meal replacement program with standardized recipes is feasible and well tolerated.

Introduction

Malignant gliomas are the commonest brain tumor in adults, with approximately 12,000 new cases annually in the United States [1, 2]. Standard therapy for glioblastoma multiforme (GBM) includes maximal feasible resection followed by radiation and chemotherapy, with bevacizumab rescue therapy for recurrence. Median survival after diagnosis is approximately 15 months [3–5]. Essentially all patients suffer recurrent disease, usually within 8 months of diagnosis. The median survival after recurrence is 25 weeks with the standard treatment of bevacizumab, with a 6 month progression free survival of 15% [5]. There is thus need for new treatment.

In the last 10 years, there has been a growing interest in alternative, metabolic treatments of GBM. [6–13] GBM cancer cells utilize aerobic fermentation of glucose in the cytosol for energy supply instead of mitochondrial oxidative phosphorylation (the “Warburg effect”) [7, 8, 10]. 18 F-fluoro-2-deoxyglucose positron emission tomography (PET) shows that human GBMs have much higher glucose utilization than normal cortex. In states of prolonged glucose deprivation, such as fasting or starvation, normal brain cells metabolize ketone bodies derived from fatty acids for energy instead of glucose. Tumor cells are poorly able to do so. They depend on glucose and glycolysis for survival. This makes tumor cells vulnerable to therapies of glucose restriction [6, 8, 10–15].

Glucose reduction and ketone exposure reduce proliferation and growth rate of human GBM cells [16] and of rodent astrocytoma lines [14]. The effect occurs independently with both glucose reduction and with ketone body exposure, with a synergistic effect between the two. Glucose deprivation results in apoptotic death in human GBM cells but not in normal cells [16]. Similarly, ketone bodies inhibit the viability of cultured human GBM cells, but not of normal cells. In mice, high circulating glucose levels accelerate tumor growth and angiogenesis and prevent apoptosis [17, 18]. Reduction in circulating glucose and increase in ketone levels through ketogenic diet (KD) with caloric restriction have pro-apoptotic, anti-angiogenic and anti-inflammatory effects, reduce expression of mTOR effector in mice with experimental malignant gliomas, reduce rodent tumor growth and tumor size, and increase survival of animals [15–17, 19–21]. In a mouse model of malignant glioma treatment with 4:1 KD, KD increased median survival by 22% from 23 to 28 days [22]. Remarkably, treatment with KD given together with radiation resulted in complete tumor remission in 9/11 animals. KD treatment lasted for 101 days. Animals remained tumor free until they were sacrificed at 299 days [22]. Calorically restricted KD combined with the glutamine antagonist, 6-diazo-5-oxo-L-norleucine (DON) appears similarly effective in 2 mouse models of orthoptic implanted GBM [21].

In patients with GBM, hyperglycemia is associated with shorter survival [23]. There have been seven reports of KD treatment of patients with GBM: 3 of 1–2 patients and 4 with 9–20 patients [24–30]. Outcomes in the anecdotal cases were better than expected [24, 25, 29]. However, in the larger studies no positive findings were reported [26–28, 30, 31]. These studies have varied widely. They have ranged in treatment duration from 6–14 weeks, in disease stage from adjunctive treatment with standard initial care to recurrent GBM, in diet design CH content from 60 g CH/day or 25% of calories derived from CH to 4:1 KD [26–28, 30], in fat content, and in presence, absence and degree of caloric restriction.

Studies with > 2 patients reported difficulty with doing the diet and, in 3/4 studies, continuing it beyond 3 months. In these studies, the diet duration was limited to 6 weeks-3 months because the investigators thought that patients could not tolerate longer periods. In one study, treatment continued for 14 weeks, but increased CH content after 8 weeks and required “intense counseling” [30]. There are three main problems with the “classic” 3:1 or 4:1 ketogenic diet: (1) it is complicated to do; (2) it is not palatable; and (3) it is done individually by each patient, and thus differs between patients in a study, and between studies.

To address these challenges, we developed a novel program of total meal replacement (TMR) of ready-made 4:1 and 3:1 KD meals to simplify and standardize the diet in order to make it easier to do and adhere to, and to make it uniform across a study and comparable between studies. The program delivers patients ready made meals using a large palette of our own recipes in a TMR program with 4:1 KD with 10 gm CH and 1600 kcal/day, with no other food consumed. The goal of this pilot study was to evaluate feasibility, safety, and tolerability of GBM treatment with this program, and to obtain pilot data on efficacy of KD treatment of GBM with this program.

Methods

This was a prospective open label study. The initial protocol was for treatment of recurrent “terminal” GBM after resection, radiation, temozolomide, and failed rescue therapy with bevacizumab. Because of good tolerance and good response of the first subject, a second study was started for adjunctive KD treatment early in the disease, concomitant with initial radiation and temozolomide therapies. The evaluation and treatment protocols of both studies were identical. Because of slow recruitment into both studies, we report the combined treatment of both protocols. Both protocols were approved by the institutional review board of Holy Cross Hospital, Silver Spring, MD. All subjects signed IRB-approved consent form. The study was conducted at the Mid-Atlantic Epilepsy and Sleep Center, Bethesda MD, and was registered as NCT01865162 (recurrent GBM) and NCT02302235 (newly diagnosed GBM).

Study Design

This was an open label Phase 1 study treatment of adults with GBM for 6 months with 4:1 [fat]:[protein + carbohydrate] ratio, 1600 kcal/day diet. Treatment was initiated either early in the disease, with initiation of radiation and temozolomide therapy (group 1) or following recurrence (group 2). Inclusion criteria, other than disease stage, evaluations and treatment protocols were identical for both groups. Primary outcomes were feasibility, safety and tolerability; secondary outcome was efficacy. Primary outcome measures included, for feasibility (1) retention in the study; for safety, (2) treatment emergent adverse events (TEAEs) and (3) treatment discontinuation because of TEAEs. Secondary outcome measures were, for efficacy, (4) overall survival time from treatment initiation, and (5) time to MRI progression. Other outcome measures included treatment compliance, hunger scale scores, fasting serum glucose and BOH levels and urine ketone levels.

Subjects

Were men and women aged 18–65 with histologically confirmed GBM of either early stage (after initial surgery/biopsy) or late stage (recurrence or progression after radiation and temozolomide treatment). Patients in group 2 had to have measurable contrast-enhancing progressive or recurrent GBM by MRI imaging. Exclusion criteria included Karnovsky Performance Score < 70, anticoagulation treatment with coumadin \geq 1 mg/day, history of non-glioma malignancy for \geq 2 years, history of uncontrolled hyperlipidemia, renal calculi, hyperuricemia, mitochondrial disease, disorders of fatty acid metabolism, porphyria, carnitine deficiency, pancreatitis, and presence of any other unstable illness.

Evaluations

Screening: Pathology of the GBM was confirmed by neuropathology review. Patients with recurrent GBM had to have MRI-documented tumor progression or recurrence. Baseline laboratory studies included serum electrolytes, renal and liver functions, CBC, PT/PTT, fasting blood glucose (FPG), and serum lipid profile (cholesterol, triglycerides, high-, low density lipoprotein, [HDL, LDL]), uric acid levels and serum b-hydroxybutyrate (BHB).

The subject’s known food allergies and special (e.g., religious) dietary requirements were reviewed. Participants were taught to measure urine ketone body (KB) levels using Ketostix (Bayer AG, Germany) which measures acetoacetate, and blood for glucose and ketone levels using self-administered Precision Xtra® Meter (Abbot Diabetes Care, Alameda, CA, USA) which measures BHB. These were done fasted in the morning and 2 hours post-prandially in the evening. Subjects were instructed to keep urine ketone/blood glucose and ketone diary.

Subsequent evaluations

included face to face visits on treatment days 7, 14, 28 to review possible early AEs, and for further education about the diet, then monthly for the 6 months of treatment and post-treatment months 6–12, and quarterly afterward, either face to face or by telephone. With 2 subjects some visits occurred via Skype because of the subjects’ long distance from the site. During each visit AEs, Karnovsky Performance Score, treatment compliance, issues with KD, urine ketone body and blood ketone and glucose diaries were reviewed. Hunger was evaluated using 7-point Licker scale (no hunger - extremely hungry). For patients with > 5% BMI loss caloric restriction was stopped. Caloric supplementation required to stay weight-neutral was calculated, with instructions to add extra calories using 100% fat-containing calories such as olive oil or 100% fat dairy produce. Baseline laboratory evaluations were repeated at treatment months 1, 2, 3, 4, and 6. Blood was drawn at 8 am, following an 8 hour fast. MRI of the brain was performed before starting treatment (baseline), and at treatment months 2, 4, and 6, and every 2–3 months after that.

Treatment diet

KD consisted of 4:1 [fat]: [protein + carbohydrate] ratio by weight, with 10 g CH/day, and with 1600 kcal restriction. Patients who did not tolerate the 4:1 ratio could choose 3:1 ratio with 20 g CH/day. The diet was supplemented with vitamins, calcium and phosphorus supplements to meet the requirements of US Dietary Reference Intakes (DRI) standard. The program consisted of 5 meals/day (breakfast, morning snack, lunch, afternoon snack, dinner), different for each day of a 2 week cycle, with repeating cycles. All meals were prepared using designed recipes (Anemone LLC, Bethesda, MD). All participants received the same meal plan but with recipe adaptation to allow personal or religious dietary restrictions (vegetarian, n = 1, no pork, n = 1) with the same caloric and macronutrient composition. Meals were prepared uniformly by one catering facility and were delivered frozen once a week. Participants were counseled not to eat any other food or and drink only 0 calorie beverages. 1 patient administered the diet on his own after the first two months, using the same KD parameters.

Medication adjustment

For subjects on steroids (n = 5), attempts were made to taper off steroids as quickly as clinically feasible.

Compliance

Compliance with the diet was evaluated at each visit by reviewing patients' consumption of food supplied by the study, extra food consumed instead of or in addition to study food, subjects' urine and blood ketone diaries and monthly serum β -hydroxybutyrate levels. It was scored as a composite of these factors on a 0–3 scale as 3 = complete compliance, 2 = partial, substantial compliance, 1 = partial, slight compliance, and 0 = complete non-compliance.

Statistical analysis

Only descriptive statistics were used because of the small sample size.

Results

Enrollment, Subject disposition and feasibility

Eight subjects were enrolled between June 2014–April 2019 (2 women, mean age 49.8 years (range 40–64) (Table 1). The study planned to enroll 6 subjects with recurrent GBM and 20 subjects with newly diagnosed GBM. Recruitment was slow resulting in early study termination. Six subjects were self-referred via the web (clinicaltrials.gov), 1 each were referred by a neurosurgeon, and by an oncologist. Additional 27 eligible subjects declined participation after screening. Ratio of eligible screened: enrolled was 3.37. Reasons for non-participation after screening included discouragement by treating oncologists (n = 11), unwillingness to undergo food restriction (n = 8) or alcohol abstinence (n = 2), and distance from the site (n = 6).

Table 1
Demographics and disease characteristics

Gender (M, %)	6 (75)
Age, years, mean (range)	49.8 (40–64) 40s: n = 5 60s: n = 3
Race	Caucasian/white: 5 Caucasian/Hispanic: 1 Asian Indian: 2
Group ¹	Group 1: 4 Group 2: 4
GBM Primary/Secondary	Primary: 6 Secondary: 2 (both in Group 2)
IDH 1 ² mutational status	Negative: 6 Positive: 1 Unknown: 1
MGMT ³ promoter methylation status	Negative: 5 Positive: 2 Unknown: 1
¹ Group 1 = newly diagnosed GBM, KD concurrent with radiation and temozolomide; Group 2 = recurrent GBM	
² IDH = isocitrate dehydrogenase	
³ MGMT = O6-methylguanine methyltransferase promoter methylation	

Subject demographics and disease characteristics are shown in Table 1. Four subjects had newly diagnosed GBM (group 1), 4 had recurrent GBM (group 2). All patients in group 1 had primary GBM; 2/4 patients in group 2 had primary GBM, 2/4 had secondary evolution of grade 3 astrocytoma to GBM. Five subjects completed the 6 months of treatment per protocol (PP), 4/4 subjects in group 1 and 1/4 in group 2. Three subjects

discontinued the study early: 2 (25%) due to disease progression, at 0.2 and 5 months; and 1 (12.5%) because of diet restrictiveness, at 1 month. Four subjects, all newly diagnosed, chose to continue KD on their own after completion of the 6 months' protocol treatment. Three of them continued KD until shortly before death, for a total of 26, 19.3 and 7 months. All deceased patients were followed until death. One patient is still alive and continuing with the diet for a total of 8 months to-date.

Safety and tolerability

KD was well tolerated. No patient discontinued the diet because of TEAEs. All six patients treated for > 1 month experienced weight loss, ranging from 11–18 lb (BMI reduction range 1.9-2 kg/m²) and asked to stop caloric restriction. Two were overweight at diet initiation, three had normal BMI. Six patients experienced hunger which abated with caloric increase. Three patients had transient nausea, in two cases associated with rescue BCNU therapy. 2 patients had transient dizziness, and one patient each had fatigue and constipation. All TEAEs were mild to moderate. There were no treatment-emergent SAEs. Seven patients died, all of GBM. Four patients complained about the restrictiveness of the diet, including 1 who stopped the diet early and three who completed the 6 months of treatment, including two who continued the diet on their own after completion of the 6 months' study protocol.

Labs

Serum ketone, glucose and lipid levels and urine ketone levels are shown in Tables 3 and 4. Cholesterol and triglyceride levels increased by > 20% in 3 patients each and declined by > 20% in one patient each. HDL levels increased by > 20% in one patient. LDL levels increased and declined by > 20% in 2 patients each. Changes in remaining patients were slight in either direction.

Table 3

Mean serum beta hydroxy butyrate (BHB) (monthly), urine ketone levels (daily) and fasting plasma glucose (FPG, daily). All figures are given to the nearest two decimal points.

Subject #	Group #	Serum BHB mmol/l		Urine Ketone levels mg/dL		FPG mg/dL	
		Baseline	KD Mean (min,max) ¹	Baseline	KD Mean (min, max) ¹	Baseline	KD Mean (min, max) ¹
1	1	2.97 ²	2.51 (1.3–4.6)	80	58.6 (15–160)	76	76.91 (49–163)
2 ³	1	NA ⁴	2.33 (1–3)	0	68.23 (0–80)	103 ³	101.35 (77–162)
3	1	0.07	3.66 (1.9–5.3)	0	64.3 (15–160)	73	85.82 (67–112)
4	1	0.95	3.14 (1.5–6.54)	0	63.66 (5-160)	80	79.86 (64–98)
5	2	NA	0.28 (0.1–0.5)	0	4.7 (0–15)	82	95.90 (73–108)
6	2	0.36	1.08 (0.06–2.11)	0	37.92 (5–80)	106	105.46 (44–174)
7	2	0.14	NA	0	NA	59	NA
8	2	NA	0.26 (0.08–0.4)	0	24.69 (5–40)	117	75.15 (65–94)
Mean Grp 1		0.51 (0.07–2.97)	2.91 (2.33–3.66)		63.69 (58.6-68.23)	83 (76–103)	86.98 (76.91-101.35)
Mean Grp 2		0.25 (0.14–0.36)	0.54 (0.26–0.28)		22.43 (4.7-37.92)	91 (59–117)	92.17 (75.15-105.46)
¹ Minimum and maximum values during the whole study							
² Patient had started KD previously on his own. BHOB was collected after screening.							
³ Patient had type 2 diabetes on oral hypoglycemic medications. He stopped all diabetic medications by KD week							
3. Baseline FPG was on Metformin 1500 mg/d, sulfonylurea 40 mg/d, all KD FPGs are off diabetic medications.							
⁴ NA = not available							

Table 4

Fasting lipid profiles, baseline and end of KD treatment. All figures are given to the nearest two decimal points. Figures in parentheses indicate range.

Subject #	Group #	Cholesterol total			TG			HDL			LDL		
		Baseline	KD	% change	Baseline	KD	% change	Baseline	KD	% change	Baseline	KD	% change
1	1	207	242	17	64	61	-5	55	75	36	140	155	11
2	1	128	313	145	82	136	66		82	NA		200	NA
3	1	303	282	-7	146	128	-12	41	48	17	232	207	-11
4	1	188	229	22	34	38	12	107	89	-17	74	132	78
5	2	306	232	-24	67	33	-51	119	101	-15	174	124	-29
6	2	257	235	-9	122	251	106	92	96	4	141	89	-37
7	2	178		NA	76		NA	45		NA	118		NA
8	2	188	225	20	108	177	64	95	91	-4	71	99	39
Mean Grp 1		206.5 (128–303)	266.5 (229–313)		81.5 (34–146)	90.8 (38–136)		67.7 (41–107)	73.5 (48–89)		107 (74–232)	173.5 (132–207)	
Mean Grp 2		207.7 (178–306)	230.7 (225–235)		93.3 (67–122)	153.7 (33–251)		87.8 (45–119)	96 (91–101)		126 (71–174)	104 (89–124)	

Efficacy

Seven subjects died. One subject is alive, completed the 6 months' treatment protocol and is continuing with the diet on her own. Only the 7 deceased subjects are included in efficacy analysis. KD treatment duration, times from diagnosis to KD initiation and to death, from treatment initiation to death and to MRI progression, and from treatment end to death are shown in Table 2. Mean survival time from diet initiation to death was 20 months for Group 1 (range 9.5–27), and 12.8 months for Group 2 (range 6.3–19.9 months). Mean survival from the time of diagnosis till death was 21.8 months for Group 1 (range 11–29.2) and 25.4 for Group 2 (range 13.9–38.7). The unusually long survival in group 2 is largely due to two patients who had secondary GBM developing from grade 3 astrocytoma, whose survival times from GBM diagnosis were 19 and 38.7 months. Mean time from KD initiation to MRI progression was 3.4 months for Group 1 (range 1.6–7.1 months), and 3.9 months for Group 2 (range 0.6–9.1). One patient with recurrent GBM who had failed bevacizumab and 3 other experimental protocols before KD had tumor progression one week after starting KD and stopped treatment. The number of subjects was too small to analyze survival parameters by age or gene typing.

Table 2

Survival and progression-free survival from diagnosis, and parameters of KD initiation, KD duration during and after protocol treatment, time from KD start- and KD end till death (all in months) and KD compliance. Only 7 patients who died are included in the analysis. Group 1: newly diagnosed GBM, with KD adjunctive treatment to XRT and temozolomide. Group 2: Recurrent GBM, with recurrence/progression after XRT, temozolomide, and recurrence/continued progression on Bevacizumab. All months are given to the nearest decimal point.

Subject #	Group #	Age	Overall survival ¹	Dx-KD start	Study RX duration	KD continued after study	KD start-death	KD start-MRI progression	Compliance ²
1	1	62	29.2	1.9	6	20	27	7.8	3
2	1	64	11	1.4	6	1	9.5	1.6	3
3	1	40	25.1	1.7	6	13.3	23.4	3.9	3
4	2	48	19	1.5	1	0	17.5	7.1	0
5	2	62	38.7	31.7	5	0	6.3	2.2	2
6	2	41	13.9	1.9	0.2	0	7.5	0.6	NA
7	2	41	30.1	1.7	6	0	19.9	3.8	2
Mean Grp 1		55.3	21.8 (11-29.2)				20 (9.5-27)	3.4 (1.6-7.1)	
Mean Grp 2		48	25.4 (13.9-38.7)				12.8 (6.3-19.9)	3.9 (0.6-9.1)	
¹ Overall survival = survival from diagnosis									
² Compliance is rated on a scale of 0-3: 0 = none, 1 = partial-slight, 2 = partial-substantial, 3 = complete									

Steroids

Five subjects were on steroids at the time of KD initiation. Two, both newly diagnosed, stopped it the day of KD initiation. They had no edema off steroids during radiation and temozolomide treatment. One patient with recurrent GBM and prominent edema tapered off dexamethasone from 8 mg within 3 weeks of starting KD, then re-started it at 2 mg/d at the insistence of her oncologist. During this time, her edema resolved. One subject continued with 2 mg dexamethasone throughout the treatment at the advice of the treating oncologist. One subject stopped dexamethasone with KD initiation, had tumor progression 2 months later and restarted dexamethasone.

Compliance

was complete (3 on a scale of 0-3) throughout the study in 4/8 patients; partial, substantial (2/3) in 1 patient throughout the study and in one patient for 3 months, after which it became partial slight (1/3); slight (1/3) in 1 patient; and not evaluable in one patient who stopped the study after 1 week because of disease progression. Most non-compliance consisted of extra food intake.

Case reports

1 subject each with new onset and recurrent GBM groups had unusually positive courses

Case 1

(Subject #8, Table 2): A 41-year old previously healthy woman presented with acute obtundation and vomiting due to left temporo-parietal GBM. She underwent "complete" resection, followed by radiation and temozolomide. The tumor recurred 8 months after diagnosis, with somnolence, dysphasia, memory impairment, right homonymous hemianopsia, right-sided body neglect, 4/5 right hemiparesis, increased MRI size of the original tumor and a new adjacent daughter lesion with surrounding edema. Bevacizumab was started together with dexamethasone. Symptoms and MRI lesions progressed. She started 4:1 10 g CH, 1600 kcal/day diet 10.2 months after initial presentation and continued with bevacizumab. She tapered off dexamethasone from 8 mg/day over the next 3 weeks, then had it re-started at 2 mg/day by her oncologist without clinical or radiological change. Six weeks after diet initiation her symptoms began to improve and resolved almost completely by 8 weeks, except for right upper quadrant anopsia. MRI showed improvement of edema and reduction of tumor size. 4 months after KD initiation MRI showed a small, clinically asymptomatic increase in tumor size compared to 1 month earlier, although the lesion was still smaller than at KD initiation. BCNU was added to bevacizumab and KD. MRI lesion size stabilized. She completed 6 months of KD study and elected not to continue it due to dietary restriction (she was Asian Indian and a vegetarian). She was asymptomatic and radiologically stable on continued BCNU and avastin for 11 months after stopping the diet, with excellent quality of life. She then developed a new lesion, refused further treatment, declined rapidly, and died 3 months later, 20 months after starting and 14 months after stopping KD.

Case 2

(Subject # 1, Table 2): A 62-year old previously healthy man started the diet 2 months after MRI diagnosis of right temporal GBM, with 95% resection. KD was started with radiation and chemotherapy. He was strictly adherent with the diet. Mean FPG dropped from 76 mg/dl at baseline to 66.75 mg/dl during treatment, with mean serum BOHB of 2.51 (1.3-4.6). He lost 12 lb in weight during the first month and increased caloric intake from 1600 kcal/day to 2200 kcal/day. He elected to continue with self-administered 4:1 KD with 20 g CH restriction after completing the 6 months of

study treatment. He had radiological progression 7.7 months after KD initiation but remained clinically asymptomatic. He continued with KD (strictly adherent) as well as a motley of supplements. He remained clinically asymptomatic with excellent quality of life until 2 months prior to his death 27 months after KD initiation, 29.2 months from diagnosis.

Discussion

The main finding of this pilot study is that prolonged (6 months) treatment of patients with GBM with a rigorous, 4:1 ketogenic diet is feasible and well tolerated when delivered as a total meal replacement program using standardized recipes and ready-made meals. To our knowledge, this is the longest KD treatment study of GBM patients to-date, outside of two isolated cases [25, 29]. It is the second study, other than one case report, to offer the most restrictive, "classic" 4:1 KD, which was used in our study for 26 weeks, 18 weeks longer than previously [30]. It is the first cancer ketogenic diet study in which all food intake was standardized and the same for all subjects.

Feasibility

While preclinical data suggests great promise for KD treatment of GBM, few clinical studies have been done [24–31]. Part of the reason is the difficulty of doing KD. KD poses three main challenges: lack of palatability, the complicated nature of meal preparation, and lack of standardization. Of the four published KD GBM studies with > 2 patients, three concluded that KD cannot be tolerated for more than 6–12 weeks [26–28], and that the maximally tolerated diet is 3:1 with 20 g CH. In a German study of restrictive calorie ketogenic diet in end-stage malignant tumors only 5/16 subjects completed the 3-month treatment period [32]. The very high fat 4:1 KD is the most difficult ketogenic diet. Recently 9 newly diagnosed GBM patients were treated with 4:1 liquid formula KD (without caloric restriction) for 8 weeks. They tolerated it well but after 8 weeks the diet was liberalized to 1.5-2:0 [fat]: [CH + protein] ratio for the 6 remaining weeks of the 14 weeks of treatment [30]. Even so, the patients required "intense counseling" to execute the diet. Furthermore, evaluation of results of KD treatment is difficult because the diet as currently practiced is individualized by each patient, making compliance evaluation and comparisons across patients within a study and between studies difficult.

In the present pilot study, we overcame these difficulties by providing the diet as a complete ready-made meal delivery program which was the same for all subjects (with adaptation for religious preferences and food allergies). This novel approach offers standardized treatment for all subjects; simplicity of execution for patients; and ease of monitoring compliance (patients either only eat food delivered, or add/substitute for it). We used the most challenging of ketogenic diets, the 4:1 10 g CH /day variant in which 90% of calories are derived from fat. The study shows that such an approach is feasible. Five of eight subjects completed the 6 months of treatment per protocol, 2 discontinued it because of tumor progression, and only 1 (12.5%) because of diet restrictiveness. Four subjects continued KD after completing the study protocol, for up to 26 months. This suggests that the ready-made TMR version of the diet may be easier for patients to do and adhere to.

Safety

The diet was well tolerated. No subject discontinued because of TEAEs. The main side effects were weight loss and hunger. The diet was calorie-restricted to 1600 kcal/day, but this restriction was abandoned in all 6 subjects treated for > 1 month because of weight loss. Hunger resolved with caloric increase. All other side effects, nausea, dizziness, fatigue and constipation, were mild and transient. Blood lipids were affected. Cholesterol and triglyceride levels increased markedly in two and three patients, respectively; HDL levels increased notably in 1 patient.

Efficacy

The small study sample precludes conclusions about KD efficacy. Two of 8 cases had an unusually favorable survival. Among possible explanations is the possibility that the anti angiogenic properties of KD may have enhanced the activity of Bevacizumab used as a second line therapy, contributing to the longer than expected survival.

Comparison with prior studies and treatment duration

Seven previous studies of KD treatment of gliomas have been reported. Three of them were 1–2 patient case reports, all showing promising results [24, 25, 29]. None of the larger studies showed the remarkable treatment response seen in the case reports and in the one patient in our study. However, the larger studies were all done for a short period of time, with less rigorous KD, with less fat and more carbohydrate content. Louw et al. used 4:1 liquid KD but only for 8 weeks, after which the diet was switched to 1.5-2:1 ratio [30] in which only ~ 60–75% of calories are derived from fat. In experimental setting, efficacy is linked to the more restricted diets such as the 4:1 diet. Even with such diets, however, glucose deprivation and ketone body exposure by themselves only retard proliferation and slows tumor growth in animals but do not kill all tumor cells [16]. It is therefore likely that a short KD course may at best retard GBM growth with re-growth following KD withdrawal. Longer treatment duration, e.g. 1 year or longer may be needed. There is at present little pre-clinical or clinical data to indicate how long KD treatment should last. We treated patients for 6 months, which is longer by almost 3 months than any previous study other than case reports [26–29]. Four of our subjects continued KD on their own past the protocol duration, including one each for total of 26 and 19 months. This was relatively easy because during the 6 months of TMR they learned the meals to cook. In epilepsy, 4:1 and 3:1 KD with CH of 10–20 g/day is sometimes continued for many years [33–35]. Our study shows such treatment is feasible in GBM patients also when TMR program is used.

Timing of intervention and its potential impact on efficacy

All our newly diagnosed patients started KD after surgical resection, in conjunction with initiation of radiation and temozolomide therapy. There was an approximately 6–8 weeks lag between diagnosis and KD initiation. It has been suggested that KD should be started before surgical resection and before radiation and temozolomide to allow maximal impact of KD [14]. This did not occur in our patients. It is possible that the time lag may have

impacted KD efficacy [29]. The most remarkable animal study of KD in malignant glioma showed cure in animals treated with 4:1 KD concurrently with radiation treatment [22]. This was done by Louw et al. who started 4:1 KD 2 weeks before radiation and chemotherapy, albeit after surgery, continued it for 8 weeks, and then switched to 1.2-2:1 KD for further 6 weeks. Six of 9 patients completed the protocol and 4 patients continued KD after the 14 weeks of study treatment. The median survival of 12.8 months was unremarkable [30]. Future studies may, therefore, consider study starting KD before surgery [29].

Limitation

The study has significant limitations. It is very small, has two different GBM patient populations (newly diagnosed and recurrent), is open label, and had potential for patient self-selection bias. KD is not a recognized treatment for GBM. Participants were all patients who were seeking additional treatment options, and were highly motivated. Concomitant treatments were allowed. The two subjects with longer than expected survival used other therapies (avastin, with pre-KD progression, but continued with KD and BCNU, started 4 months after KD in one patient, and a motley of supplements in the other patient). Factors that may affect survival such as age, genetic background and concomitant treatments were not controlled. The TMR was “one size fits all”, without caloric adjustment for gender, size or activity. We are adapting the program to allow such flexibility in future.

Recruitment

In addition, we failed to recruit the planned number of subjects. During almost 5 years of recruitment, only 8 subjects were recruited. A much larger number of eligible patients was screened, with eligible screened: enrolled patient ratio 3.4:1. Reasons for non-enrollment varied. The largest was discouragement by treating oncologists and competing studies by neurosurgeons. We made a large recruitment education effort targeted at local oncologists and neurosurgeons, with little success and only two referrals. The second largest reason was patients’ unwillingness to restrict the pleasures of food and alcohol during the perceived short remainder of their life. This is a major limitation. It may not change unless and until KD treatment is shown to improve outcome.

Caloric restriction

Animal evidence suggests that caloric restriction may have an additional benefit in KD [7, 8]. This has led to proposals that KD in GBM be calorically restricted, for example to 600–1200 kcal/day, supported by a couple of remarkable case reports [25, 29]. Our study shows that caloric restriction may not be feasible, except in exceptional individuals. Our diet was only modestly calorically restricted at 1600 kcal/day, and yet it was associated with weight loss (and hunger) in all subjects treated for > 1 month. This was unacceptable to patients, families, and treating oncologists, leading to caloric increase to prevent further weight loss.

Steroid reduction

Steroids are used routinely in GBM for treatment of cerebral edema. They elevate blood glucose. Hyperglycemia is associated with reduced survival in GBM patients [23] and in animals [17], and with worse functional outcome after surgical resection in GBM patients [36]. We were able to withdraw (n = 2) or reduce (n = 1) the dose of dexamethasone in 3/5 patients who were taking it before KD. In two, there was no edema during the ensuing radiation and temozolomide treatment. In another, prominent edema resolved after KD initiation even as the dexamethasone dose was tapered. This has been reported previously [25, 28, 29]. KD reduces vascular permeability and edema in a mouse glioma model [9]. Substitution of steroid therapy for edema with KD and the associated avoidance of steroid-induced hyperglycemia may provide benefit independent of KD effect on the tumor cells and should be further evaluated.

Compliance

Compliance is an issue with any diet, more so in patients with terminal illness. However, our study suggests that complete ready-made cooked meal replacement may be conducive to compliance: 4/7 patients treated for > 1 week had complete compliance, 2 had substantial compliance, and only 1 had poor compliance.

In summary, this pilot study shows that long term rigorous ketogenic diet treatment of patients with malignant cancer such as GBM is feasible and well tolerated with the use of a standardized total meal replacement program using ready-made cooked meals. If issues with recruitment can be resolved, this approach allows testing of KD in GBM and other malignant cancers in a standardized way on large cohorts of patients.

Declarations

Ethics approval and consent to participate: The study was approved by the institutional review board of Holy Cross Hospital, Silver Spring, MD. All subjects signed IRB-approved consent form. The study was conducted at the Mid-Atlantic Epilepsy and Sleep Center, Bethesda MD, and was registered as NCT01865162 (recurrent GBM) and NCT02302235 (newly diagnosed GBM).

Consent for publication: Not applicable

Availability of data and materials: The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests: PK is the founder and CEO of Anemone, LLC which provided recipes used in the study. None of the other authors have any competing interests.

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Authors' contribution:

PK designed the study, collected, analyzed and interpreted the data and wrote the manuscript

IT assisted with the design of the study, collected, analyzed and interpreted the data and contributed to writing and editing the manuscript.

GZ assisted with the design of the study, contributed to interpretation of the data, and to writing and editing the manuscript.

AT contributed to the analysis and interpretation of the data, and to editing the manuscript.

JCM assisted with the design of the study, contributed to interpretation of the data, and to editing the manuscript.

All authors read and approved the final manuscript.

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