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Treatment of Multiple Glioblastoma with Herbal Medicines

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Abstract

Although it has been proven that the active metabolite of plants from the genus *Artemisia L.* can pass through the blood-brain barrier, so far only one paper has been published on the successful use of herbal medicines, which include the aforementioned plant, in the treatment of glioblastoma. The patients took five types of herbal medicines (in the form of tea) on a daily basis at regular intervals. The preparations were in combination with oncological treatment and continued to be used after the oncological treatment was completed. In subjects with tumour progression, the composition of the herbal medicines was modified. The results of the treatment were monitored by scanning the affected site by nuclear magnetic resonance technique or evaluated on the basis of the patients' current need for corticosteroids. In the first two patients, whose treatment with temozolomide was discontinued, the growth of the larger (symptomatic) tumour was haltedafter the introduction of phytotherapy, and the regression of secondary deposits also occurred. In the remaining 3 subjects,who had been treated with a combination of temozolomide and phytotherapy, there was a reduction of tumour size, and it should be emphasized that, after 33 months of phytotherapy, the fourth patient showed a complete regression of all tumour lesions. The lack of a clear strategy for the treatment of multiple glioblastomas, as well as the limited number of therapy options, are the reason for the very modest results achieved today in the treatment of these tumours. The results presented in this paper show that phytotherapy has its place in the treatment of glioblastoma presenting with multiple lesions at the time of diagnosis.

Keywords: Phytotherapy, Multiple Glioblastomas, Temozolomide, Nuclear Magnetic Resonance

List of abbreviations: DHA: dihydroartemisinin**;** PT: phytotherapy**;** GBM: glioblastoma multiforme**;** IDH 1: isocitrate dehydrogenase 1**;** MGMT: O6-methylguanine-DNA methyltransferase**;** NMRI: nuclear magnetic resonance**;** PTS: phytotherapy of salvation; RT/CT: combined radiochemotherapy**;** StPT: standard phytotherapy; TMZ: temozolomide

Introduction

Previously, it was considered that glioblastomas (GBMs) presenting with multiple lesions at the time of diagnosis were rather rare, appearing in 0.5-20% of cases [1]. However, recent research suggests that multiple lesions can, by using nuclear magnetic resonance imaging (NMRI), be detected in approximately 35% of affected persons at first scanning. The increase in the number of patients diagnosed with multiple GBMs was due to the rapid development of new MRI sequences and imaging techniques, thus increasing the reliability of this diagnostic method in the detection of tumour lesions of the brain [2]. Multiple GBMs can be divided to multifocal and multicentric. In the case of multifocal tumours, there is a clear path of dissemination, while in multicentric tumours the manner of dissemination cannot be precisely determined. It is considered that glioblastomas presenting with multiple lesions represent a more malignant type of tumour that is more inclined to migration and therefore has a very poor prognosis [3]. The average survival rate of the patients is shorter than of those with solitary GBM, approximately 3 months [2].

The modest results that medicine has achieved in the treatment of GBM and some other malignant tumours have triggered the development of new therapeutic strategies, including the introduction of various herbal medicines for the treatment of affected persons. Most of the reports cover the use of herbal medicines, whose composition includes plants from the genus Artemisia L., whose active metabolite, dihydroartemisinin (DHA), prevents the proliferation of tumour cells [4,5]. Once it was proven that DHA could pass through the blood-brain barrier, room for researching its action on brain tumours appeared [6]. However, so far only one paper has been published on the successful use of herbal medicines,which include plants from the genus Artemisia L.,in the treatment of GBM [7].

The aim of this paper is to demonstrate that the pharmacologically active ingredients found in appropriate herbal medicines can help patients diagnosed with multiple GBMs.

Methods

The patients which cases are presented in this research decided to apply for phytotherapy (PT) voluntarely after their doctors introduced them to the possibilities and results that modern medicine can provide conserning their diseases. Prior to the start of phytotherapy (PT), all the patients submitted their medical records containing pathohistological results including the diagnose and the genetic characteristics of the tumor, informations about the previous and current treatment and the scan results of the affected area using nuclear magnetic resonance (NMR). These data were used as the basis for a comparative monitoring of the effectiveness of PT in terms of comparing the dimensions and the number of lesions of the tumor prior to PT with the results of the control scans that patients underwent during and after PT.

The first stage of preparation of the herbal remedies consists in choosing the highest quality plants. In the selection of the plants, priority was always given to those that can be found in nature, meaning that 80 % of the plants included in the herbal remedies arewildcrafted. They have been growing without any human intervention and such plants obtained the necessary resources for growth and development on their own.This way they passed natural selection process, which makes them the finest representatives of their sort. The remaining 20% of the plants are obtained by breeding.

The next stage involves the drying of the plants. All of the plants are dried in a natural way, without bringing any additional energy sources from aside.After they have been dried, the humidity percentage of the plants should not exceed 10 % because that level of humidity ensures the plant conservation for longer periods of time. Before bringing the dried plants into the storage room, they are sterilized by rapid cooling to - 15°C. From the raw material prepared in such a manner, preparations are made just before their application. All the plants that are part of herbal medicine, whether they are wild sorts or obtained by breeding, are from Bosnia and Herzegovina.

Standard phytotherapy (StPT)

During this research the patients were treated with two different combinations of herbal medicines.The first combination was marked as standard phytotherapy (StPT). This combination of herbal medicines consisted of five types of herbal mixtures that differed in composition (preparation 1, preparation 2, preparation 3, preparation 4, and preparation 5).

The herbal remedy ingredients are given in **Tables 1-5.**

The patients prepared the herbal medicines and took them in the form of tea every day at regular intervals. The patients took preparation no. 1 at 7 a.m., no. 2 at 10 a.m., no. 3 at 1 p.m., no. 4 at 4 p.m., and no. 5 at 7 p.m. **(Table 6).**

In those patients whom progression occured and those whom had poor prognosis a combination of herbal remedies marked as pyhtotherapy of salvation (PTS) was included. This group of herbal remedies consisted of the first fourpreparations included in the composition of standard phytotherapy, while the fifth herbal preparation was not included. This herbal remedies combination was taken by the patients 5 times per day as well, drinking theno. 1 herbal preparation twice per day at 7 a.m. and 7 p.m., and the no. 2,3,4 once per day at 10 a.m., 1 p.m., 4 p.m. Hereby, the daily dose of preparation no. 1 was doubled **(Table 6).**

All of the preparations consisted of grinded plant parts only. The plants included in their composition were grinded to a standard level. For the flowers, stems and leaves a sieve no. 6 was used (raw cutting), for the roots and barks a sieve no. 3, for seeds and fruits a sieve no. 2 (fine cutting) [8]. All of the preparations were made the same way by the patients, for one dose of tea, 1,5 g of herbal mixture and 200 cm^3 of water were needed.

During the follow-up, i.e., when comparing the condition of patients before and after PT, the following key indicators were used:

- Information on the tumor size and number of lesions;
- Information on the extent of cerebral edema;
- Information on the antiedematose therapy and dosage of synthetic corticosteroids;
- Information on the general status of the patients (Karnofsky performance status);
- Information on previous and current oncological treatment;
- Information on values of liver markers;

Besides the mentioned indicators, a record of other indicators was followed (e.g. the usage of antiepileptics, duration and side effects of PT, age of the patients ect.)

Statistical Analysis

Since five cases were presented in this study, there was no basis for drafting a statistical analysis.

Table 1. *Ingredients of preparation 1.*

Preparation 1				
Pharmaceutical name	**Botanical name**	**Family**	**Part used**	**Percentage representation**
Herba artemisiae-alba	*Artemisia absinthium* L.	Asteraceae	Herba	25%
Herba artemisiae vulgaris	*Artemisia vulgaris* L.	Asteraceae	Herba	25%
Visci albi herba	*Viscum album* L.	Santalaceae	Herba	25%
Centaurii herba	*Erythrea centaurium* L.	Gentianaceae	Herba	25%

Table 2. *Ingredients of preparation 2.*

Preparation 2				
Pharmaceutical name	**Botanical name**	**Family**	**Part used**	**Percentage representation**
Herba catariae	*Nepeta cataria* L.	Lamiaceae	Herba	20%
Melissae folium	*Melissa officinalis* L.	Lamiaceae	Folium	15%
Thymi herba	*Thymus vulgaris* L.	Lamiaceae	Herba	10%
Origani herba	*Origanum vulgare* L.	Lamiaceae	Herba	10%
Matricariae flos	*Matricaria chamomilla* L.	Asteraceae	Flos	10%
Lupuli strobili	*Humulus lupulus* L.	Cannabaceae	Storobili	10%
Rosmarini folium	*Rosmarinus officinalis* L.	Lamiaceae	Folium	5%
Calendulae flos	*Calendula officinalis* L.	Asteraceae	Flos	5%
Valerianae radix et rhizoma	*Valeriana officinalis* L.	Valerianaceae	Radix et Rhizoma	5%
Bursae pastoris herba	*Capsella bursa pastoris* L.	Brassicaceae	Herba	5%
Basilici herba	*Ocimmum basillicum* L.	Lamiaceae	Herba	5%

Table 3. *Ingredients of preparation 3.*

Preparation 3				
Pharmaceutical name	**Botanical name**	**Family**	**Part used**	**Percentage representation**
Althaeae radix	*Althaea officinalis* L.	Malvaceae	Radix	20%
Althaeae folium	*Althaea officinalis* L.	Malvaceae	Folium	20%
Betulae folium	*Betula pendula* Roth	Betulaceae	Folium	20%
Menhtae piperitae folium	*Menhta piperita* L.	Lamiaceae	Folium	15%
Herba glechomae	*Glechoma hederacea* L.	Labiatae	Herba	15%
Cheliodonii herba	*Chelidonium majus* L.	Papaveraceae	Herba	10%

Table 4. *Ingredients of preparation 4.*

Preparation 4				
Pharmaceutical name	**Botanical name**	**Family**	**Part used**	**Percentage representation**
Urticae herba	*Urtica dioica* L.	Urticaceae	Herba	20%
Millefolii herba	*Achilea millefolium* L.	Compositae	Herba	20%
Betulae folium	*Betula pendula* Roth	Betulaceae	Folium	30%
Teucrii montani herba	*Teucrium montanum* L.	Lamiaceae	Herba	15%
Centaurii herba	*Erythrea centaurium* L.	Gentianaceae	Herba	15%

Table 5. *Ingredients of preparation 5.*

Preparation 5				
Pharmaceutical name	**Botanical name**	**Family**	**Part used**	**Percentage representation**
Herba catariae	*Nepeta cataria* L.	Lamiaceae	Herba	25%
Melissae folium	*Melissa officinalis* L.	Lamiaceae	Folium	20%
Thymi herba	*Thymus vulgaris* L.	Lamiaceae	Herba	15%
Matricariae flos	*Matricaria chamomilla* L.	Asteraceae	Flos	15%
Lupuli strobili	*Humulus lupulus* L.	Cannabaceae	Storobili	10%
Rosmarini folium	*Rosmarinus officinalis* L.	Lamiaceae	Folium	5%
Calendulae flos	*Calendula officinalis* L.	Asteraceae	Flos	5%
Bursae pastoris herba	*Capsella bursa pastoris* L.	Brassicaceae	Herba	5%

Table 6*. Time of herbal remedies consumption and dosage.*

	Standard phytotherapy (StPT)				
Herbal medicine no.	***1***	***2***	***3***	***4***	***5***
Time of taking tea	*Every day at 7 a.m.*	*Every day at 10 a.m.*	*Every day at 1 p.m.*	*Every day at 4 p.m.*	*Every day at 7 p.m.*
Daily dose of tea	*200 cm³*	*200 cm³*	*200 cm³*	*200 cm³*	*200 cm³*
	Phytotherapy of salvation (PTS)				
Herbal medicine no.	***1***	***2***	***3***	***4***	***1***
Time of taking tea	*Every day at 7 a.m.*	*Every day at 10 a.m.*	*Every day at 1 p.m.*	*Every day at 4 p.m.*	*Every day at 7 p.m.*
Daily dose of tea	*200 cm³*	*200 cm³*	*200 cm³*	*200 cm³*	*200 cm³*

Results

The results of the study were given through case reports.

Case Reports

Case report of patient no. 1

Due to persistent intense headaches, weakness of the left limbs and vision problems which manifested in the periodical occurrence of double vision in a patient aged 36 years, on 24 December 2012, NMRI imaging was performed. On that occasion, a multilocular expansion in the right cerebral hemisphere was found. A larger tumour formation in the frontal region on the right side, and partially in the temporal and subcortical region, was 35 mm in diameter with a massive perifocal oedema affecting almost the entire right hemisphere (**Figure 1A**). Another oval cystic lesion was located in the frontal parasagittal region on the right, with a diameter of 15 mm (**Figure 1B**). The patient underwent surgery on 03 January 2013, and the resection of the larger, symptomatic tumour was performed on that occasion, while the smaller one was not resected due to its location. An analysis of tumour tissue samples showed that it was a multicentric propagation of glioblastoma. P53 was positive in 20% of nuclei, while the proliferative index measured by using Ki67 antibodies was 20%. Immunocytochemical staining toisocitrate dehydrogenase - 1 (IDH - 1) was negative. The Karnofsky Performance Scale Index was estimated at 70. The patient was administered antiepileptic drugs and dexomethasone at a daily dose of 8 mg.

During the period between 14 March and 10 April 2013, treatment with radiotherapy was continued, at total therapeutic dose of 45Gy in 20 fractions. Radiotherapy was administered along with the accompanying therapy with Temozolomide (TMZ), at a daily dose of 75 mg/m^2 of body surface area. After completion of the combined radio and chemotherapy (RT/CT), the patient continued monotherapy treatment with TMZ, at a dose of 150 mg/m^2 of body surface area for 5 days during each 28-day cycle. He applied for phytotherapy at the

beginning of April 2013, after finishing RT/CT. He started to use a composition of herbal medicines marked as a standard phytotherapy, which he used along with TMZ. After 3 cycles of monotherapy with TMZ and 3 months of PT, on 08 July 2013, a control NMRI was performed.

In the right frontal region of the control scan, the condition after osteoplastic craniotomy was observed, with a singled out irregular zone in the total area of approximately 64 x 50 mm. Within that area, a round, clearly limited hypodense change of approximately 30 x 27 mm **(Figure 1C)** was singled out, which had a compressive effect on the frontal horn of the right lateral ventricle. High in the frontal parietal region around, clearly defined hypodense zone, 15 x 13 mm in diameter,could be observed along with two ventral locations with diameter up to 5mm **(Figure 1D)**. A noticeable mild shift of midsagittal plane for approximately 3-4 mm could be observed. The finding showed tumour progression with satellite lesions present.

After this finding, further treatment with TMZ was discontinued, and the patient continued to use only phytotherapy that was modified; instead of the standard PT, PTS was introduced. The dose of dexamethasone remained the same. After the modification of the composition of herbal medicines, the control scan on 28 October 2013 showed that the dimensions of the tumour lesions had remained approximately the same **(Figures 1E and 1F)**. The same scan showed that there was a reduction of the volume of cerebral oedema, so the daily dose of dexamethasone was reduced to 4 mg. The next scan, performed on 03 June 2014, showed that the dimensions of the larger lesion had remained approximately the same **(Figure 1G)**, while the cystic structure located in the frontal parasagittal region on the right had smaller dimensions, and the several satellite lesions surrounding it were also smaller **(Figure 1H)**. After this finding, treatment with dexamethasone was discontinued.

The next NMRI imaging, performed on 20 October 2014, showed a change in the structure and dimensions of the lesion in the frontal parietal region on the right, where instead of one tumour structure, several small tumours surrounded by a large cerebral oedema could be observed **(Figure 1I)**. Compared to previous imaging, the total dimensions of the tumour mass were slightly larger. On the other hand, the cystic lesion located in the frontal parasagittal region on the right appeared smaller on this scan, with a diameter of approximately 7 mm, while in its vicinity only one satellite structure measuring 1-2 mm could be observed **(Figure 1J)**. Dexamethasone was reintroduced to the patient at a dose of 8 mg/day, and he continued to use the same combination of herbal medicines. Shortly after this finding, the patient's condition worsened, which manifested by frequent headaches on the right side that did not stop even after the daily dose of dexamethasone was increased to 16 mg/day. On 20 January 2015, the patient underwent another surgery. After leaving the hospital, the patient continued to use herbal medications, but his condition soon worsened again. He died in March 2015, 27 months after the initial diagnosis.

Case report of patient no. 2

After several epileptic seizures in a man aged 56 years, NMRI imagining was performed on 30 October 2014. On that occasion, two expansive tumour changes were found **(Figure 2A)**. The larger lesion (black arrows in figure 2), which was located in the temporal parietal region on the right, measured 29 x 26 x 27 mm, while the other tumour mass (white arrows in figure 2), located in the frontal subcortical region on the left in the splenium of the corpus callosum, had an elongated, stick shape of 18 mm in length. A tissue sample, obtained

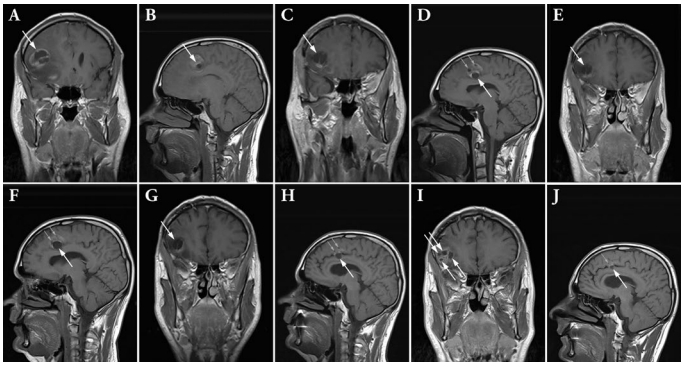

Figure 1. *Chronological representation of the NMRI findings of the patient no. 1. Tumour tissue is marked with arrows. (**A**) 24 December 2012, scan of primary (symptomatic) tumour prior to surgery. (**B**) 24 December 2012, scan of secondary tumour. (**C**) 08 July 2013, progression of primary tumour. (**D**) 08 July 2013, progression of secondary tumour with satellite lesions (marked with small arrows). (**E** and **F**) 28 October 2013, halt of the progression of both tumour lesions. (**G**) 03 June 2014, no change in size of a larger tumour. (**H**) 03 June 2014, regression of secondary tumour and satellite lesions surrounding it. (**I**) 20 October 2014 change in structure and progression of primary tumour. (**J**) 20 October 2014, further regression of the smaller tumour.*

by a stereotactic biopsy, showed that it was GBM. The methylation of the O^6-methylguanine DNA methyltransferase (MGMT) gene was not detected. The proliferative index measured by Ki67 antibodies was 20%. Immunocytochemistry of 20% tumour cells gave a positive response to p53. There was no mutation of the IDH-1 gene. During an epileptic seizure, the patient fractured his upper arm, which further aggravated his general condition. The Karnofsky Performance Scale Index was estimated at 70, and patient started taking antiepileptic drugs. On 12 November 2014, after the biopsy was performed, the second NMRI imaging was performed, which showed significant progression of both tumours. The larger tumour measured 43 x 37 x 41 mm, while the smaller tumour increased from 18 mm to 22 mm **(Figure 2B)**. In addition, the scan showed the presence of an extensive cerebral oedema, so, in combination with an antiepileptic drugs, an anti-oedema therapy with dexamethasone was administered to the patient at a daily dose of 4.5 mg. At the beginning of December, the dose of dexamethasone was increased to 9 mg per day.

After the treatment of the fracture of the upper arm, oncology treatment was started on 17 December 2014. A combined radio and chemotherapy by Stupp protocol was administered. After 33 days of daily administration of TMZ at a dose of 75 mg/m^2 of body surface area (total daily dose of 160 mg), the patient developed severe thrombocytopenia, so the oncological treatment was continued only with radiotherapy at a total dose of 60 Gy. One month after the completion of RT and after the blood count of the patient had stabilized, on 02 March 2015, monotherapy with TMZ was introduced, which was supposed to last 6 cycles of 28 days at a dose of 150 mg/$m^2$of body surface area (total daily dose of 320 mg). Upon the completion of the first cycle, thrombocytopenia occurred again, so further treatment with TMZ was discontinued. The oncological treatment was thus completed.

In mid-December 2014, the patient started with phytotherapy, combined with radio and chemotherapy. A combination of herbal medicines marked as phytotherapy of salvation (PTS) was introduced, which continually took without interruption for the following 20 months. The first control NMRI after the completion of the oncologic treatment was performed on 30 April 2015. On that occasion, progression of the larger tumour, measuring 53 x 35 x 54 mm, was established, while the

same control scan showed a regression of the smaller tumour, whose length was reduced from 22 mm to 8 mm **(Figure 2C)**. The patient continued taking the same combination of herbal medicines, and the daily dose of dexamethasone remained the same (9 mg/day). Three months later, on 27 July 2015, a new control NMRI was performed, which showed a regression of the larger tumour, measuring 46 x 34 x 31 mm at the time, while the length of the smaller tumour was 5 mm **(Figure 2D)**. The daily dose of dexamethasone was first reduced to 4.5 mg, and after one month he stopped taking anti-oedema therapy. Over the next couple of months, the patient felt well and did not experience any other discomforts except occasional dizziness.

At the beginning of November, the patient started frequently feeling weakness in his legs, so urgent MRI imaging was performed on 19 November 2015. The scan showed a progression of the tumour mass located in the temporal-parietal region on the right, measuring 61 x 40 x 53 mm, while the diameter of the tumour located in the splenium of the corpus callosum was even smaller than on the previous scan, measuring 1-2 mm **(Figure 2E)**. The patient continued to take the same combination of herbal medicines, and dexamethasone at a dose of 9 mg per day was reintroduced.

On 14 December 2015, a regular control scan was performed, which registered unchanged dimensions of the larger tumour located in the right hemisphere of the cerebrum, but there was an increase in the diameter of a smaller tumour lesion to 5 mm **(Figure 2F)**.

The control scan performed on 25 January 2016 showed that the dimensions of the larger tumour were unchanged, while the disseminate in the splenium of the corpus callosum increased to 12 mm (**Figure 2G).** The following control scan performed on 11 May 2016 showed a moderate increase in the diameter of the neoplastic process in the parietal-temporal region on the right, measuring 64 x 53 x 58 mm, while the diameter of the disseminate in the splenium of corpus callosum was 18 mm **(Figure 2H)**. The patient continued to take the same combination of herbal medications, but the dose of dexamethasone was increased to 18 mg/day.

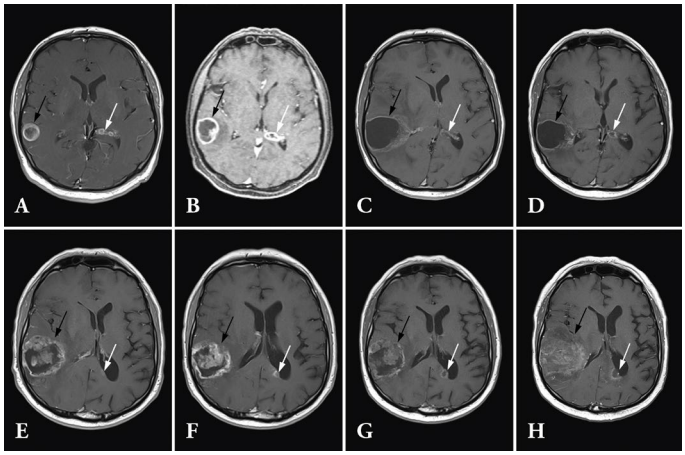

Figure 2. *Chronological representation of the NMRI findings of the patient no. 2. Tumour tissue is marked with arrows. Primary tumour is marked with a black arrow, and secondary tumour is marked with a white arrow. (**A**) 30 October 2014, scan prior to the biopsy. (**B**) 12 November 2014, scan following the biopsy. Progression of both tumour lesions. (**C**) 30 April 2015, progression of primary and a regression of secondary tumour. (**D**) 27 July 2015, regression of both tumours. (**E**) 19 November 2015, progression of primary and further regression of secondary tumour. (**F**) 14 December 2015, progression of secondary tumour (**G**) 25 January 2016, further progression of secondary lesion. (**H**) 11 May 2016, a moderate increase of both tumour lesions.*

For the next two months the patient's condition was relatively stable, and in mid-July 2016 he suddenly died of the pulmonary embolism.

Case report of patient no. 3

Due to a crisis of consciousness, which by its characteristics was consistent with an epileptic seizure, in a woman aged 37 years, on 22 December 2015 a head NMRI was performed, which established the presence of an expansive and infiltrative lesion in the medial, temporal and occipital lobe on the left, which also affected the cortex and white matter and was spreading to the right hemisphere **(Figure 3A)**. Due to the size and position of the tumour, surgery could not be performed. After a stereotactic biopsy was performed and the tissue examined by a pathologist, a diffuse astrocytoma (Gr-2) was diagnosed. Proliferativeactivity in the examined sample determined by Ki 67 was 1%. Immunocytochemical staining to IDH-1 was positive. P53 was negative. The Karnofsky Performance Scale Index was estimated at 80. The patient was administered antiepileptic drugs.

In the period from 03 March to 11 April 2016, the patient was treated with radiotherapy at a total dose of 56 Gy in 23 cycles. The control scan performed on 28 July 2016 **(Figure 3B)** still showed an expansive tumour lesion with cystic degenerations in the median portion of the temporal lobe, which affected n. amygdala, hippocampus and parahippocampal gyrus. The tumour had propagated into the median portion of the occipital lobe, affecting the splenium of the corpus callosum, and was spreading to the right hemisphere. The part of the described change that affected the corpus callosum on the left side of the corpus measured 40 x 20 mm in diameter and showed **T2 shine through effect**, and the mid-sagittal plane was shifted to the right for approximately 5 mm. In the region of central sulcus on the left a cortical lesion measuring 8 mm could be observed, corresponding to multifocal propagation of the tumour. The finding showed signs of progression and an increase of the tumour grade.

The patient started receiving anti-oedema therapy (Dexametazone 4 mg/day) in combination with antiepileptic drugs, and the oncological treatment was continued by introducing TMZ in 6 cycles of 28 days at a dose of 150 mg/m^2 of body surface area, 5 days during each cycle.

In late July 2016, the patient applied for PT and started using it in combination with TMZ. A combination of herbal medicines marked as phytotherapy of salvation was immediately introduced. The next control scan was performed on 09 January 2017, after the completion of the chemotherapy she was receiving in combination with PT. The scan showed a discreet regression of the tumour **(Figure 3C).** On the scan, the portion of the tumour that affected the corpus callosum was 39 x 18 mm (earlier 40 x 20 mm), while the previously observed lesion at the level of the central sulcus was approximately the same size as on the previous scan. The dose of dexamethasone remained the same, and the patient continued the treatment only with phytotherapy.

The next control scans performed in June and October 2017 showed an unchanged status in comparison to the scan from January 2017 **(Figures 3D and 3E)**. NMRI imaging performed on 11 May 2018 showed mild regression of the lesion located in the region of central sulcus, and the regression of the cerebral oedema has also occurred **(Figure 3F)**. After this finding, anti-oedema therapy was discontinued, and the patient continued to use the herbal medicines in full capacity.

Case report of patient no. 4

Due to headache locatedin the orbital region on the left, which lasted for 3 months, NMRI imagining was performed on a patient aged

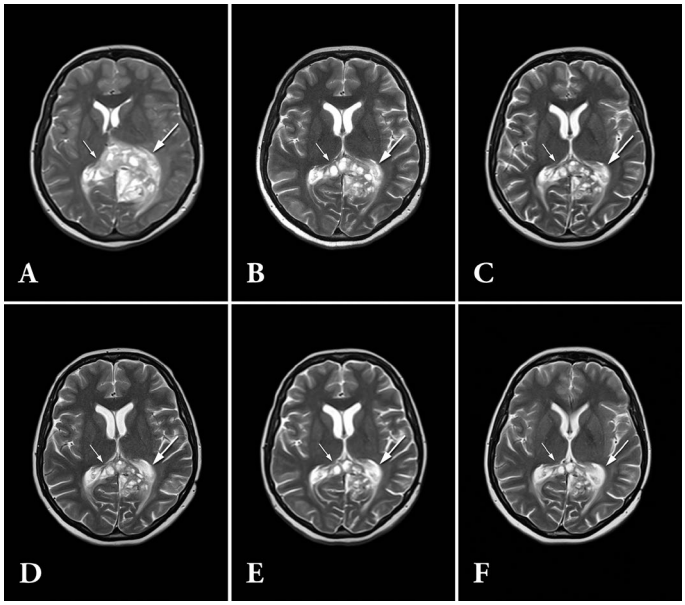

Figure 3. *Chronological representation of the NMRI findings of the patient no. 3. Tumour tissue is marked with arrows. Multifocal propagation of the tumour is marked with a small arrow. (**A**) 22 December 2015, the first scan. (**B**) 28 July 2016, progression of the grade. (**C**) 09 January 2017, discreet regression of tumour. (**D and E**) Scans from June and October 2017 show no significant changes. (**F**) 11 May 2018, regression of a small lesion.*

43 years on 29 June 2012. On that occasion, in the areas of the white matter of the temporal limb on the left, a lesion with unclear margins was observed (**Figure 4A**), which by its morphological characteristics corresponded to a diffuse astrocytoma (Gr-II). Control scans over the next two years did not show significant changes in the character and dimensions of the observed lesion, until the scan performed on 13 June 2014 established the tumour progression, measuring 9 mm (**Figure 4B**). On 14 July 2014, a stereotactic biopsy was performed, which confirmed the diagnosis of diffuse astrocytoma (Gr-II). Proliferative activity in the examined sample determined by Ki 67 was 6%. The Karnofsky Performance Scale Index was estimated at 90. The next control scan, performed on 30 January 2015, showed the continued growth of the tumour, measuring 13 mm (**Figure 4C**).

After that, in the period from 15 March 2015 to 17 April 2015, radiotherapy was performed, with total dose of 60 Gy. Shortly after the completion of radiotherapy, the patient began to complain of frequent headaches and speech disturbances. The NMRI performed on 23 July 2015 showed further growth of the tumour. The scan showed an oval nodule of good post-contrast imbibition in the upper part of the temporal gyruson the left, approximately 18 mm in dimension. Along the nodule there was a visible cystic zone of similar size **(Figure 4D).** Post-contrast, several newly formed smaller zones of imbibition of the contrast in the temporal region on the left, and suspected in the temporal region on the right were visible. After the progression was identified, the physicians opted for a surgical procedure, which was performed on 30 July 2015. A partial resection was performed. An analysis of the tumour tissue within which contained small focal necrosis showed that the tumour was a glioblastoma. Immunocytochemical staining to IDH-1 was negative, and P53 was positive in approximately 20% of nuclei. Due to oedema in the temporal region, dexamethasone was introduced at a daily dose of 4mg. The first control NMRI after the surgery, performed on 27 August 2015, showed the tumour residue (**Figure 4E**), while the zones of contrast imbibition in the temporal region on the left and on the right were larger in dimension than on the scan performed prior to the surgery (**small arrows on figures 4F and 4G**). The finding indicated a further progression of the tumour process, and the daily dose of dexamethasone was increased to 8 mg.

The oncological treatment was continued by introducing TMZ in 12 cycles of 28 days at a dose of 200 mg/m^2 (340 mg) of body surface area, 5 days during each cycle. At the same time, in combination with TMZ, the patient started to take phytotherapy. The following control scans, performed during 2016, showed a continuous reduction of tumour residue and the gradual disappearance of the zones of contrast imbibition that were initially observed in August 2015.

A control scan, performed on 10 February 2016, showed a retraction of the postoperative cavity, as well as a regression of the hyperintensity of the temporal region which was accompanied by reduced compressive effect on the lateral ventricle on the same side (**Figure 4H**). Dexamethasone was reduced to 4 mg per day, and 6 months later dexamethasone was discontinued. The control scan from 06 June 2017 established only the presence of the scar tissue from the surgery, with no signs of imbibition (**Figure 4I**). Once this fact was confirmed by the scan performed on 13 June 2018 (**Figure 4J** the patient stopped taking herbal medicines after 33 months.

Case report of patient no. 5

Due to headaches and motor weakness of the left limbs, emergency MRI imagining was performed on a woman aged 55 years on 02 November 2017. On that occasion, a large solid cystic expansion process was diagnosed in the temporal region on the right, measuring 80 x 60 x 50 mm, with multicentric propagation to the parietal lobe **(Figure 5A)**. The tumour was accompanied by an extensive perifocal oedema, and the dislocation of the lateral ventricle occurred due to its pressure. An anti-oedema therapy with dexamethasone was administered at a dose of 8 mg per day. On 24 November 2017, the patient underwent frontal-temporal craniotomy on the right side, and a maximal reduction of the tumour. Based on the analysis of the tumour tissue sample taken, the diagnosis of glioblastoma multiforme was established. Immunocytochemistry of tumour cells was negative top53 and IDH1. The proliferative index of tumour cells determined by the use of Ki67 antibodies in proliferative most active foci was 15%.

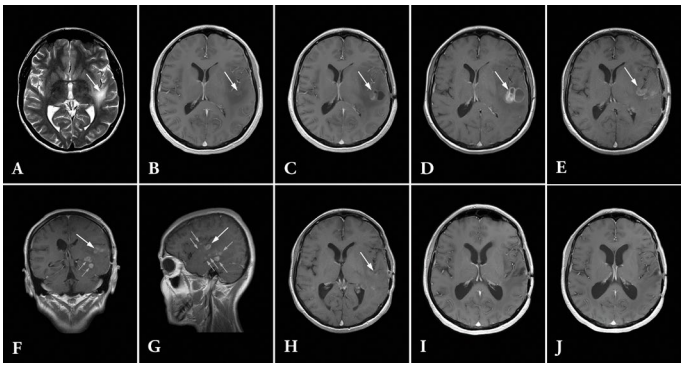

Figure 4. *Chronological representation of the NMRI findings of the patient no. 4. Tumour tissue is marked with arrows. (**A**) 29 June 2012, the first scan. (**B**) 13 June 2014, tumour diameter 9 mm. (**C**) 30 January 2015, a tumour diameter of 13 mm. (**D**) 23 June 2015, a tumour diameter of 18 mm. (**E, F and G**) 27 August 2015, the scan of residual tumour after the surgery (big arrow) surrounded by secondary lesions (small arrows). (**H**) 10 February 2016, regression of tumour. (**I**) 06 June 2017, scar with no signs of tumour. (**J**) 13 June 2018, normal finding with no signs of the disease.*

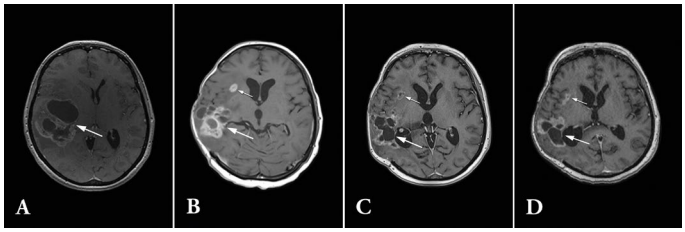

Figure 5. *Chronological representation of the NMRI findings of the patient no. 5. Symptomatic tumour is marked with a big arrow, and satellite lesions with a small arrow. (**A**) 02 November 2017, scan prior to the surgery. (**B**) 20 April 2018, progression after the surgery. (**C**) 30 July 2018, regression of symptomatic tumour and satellite lesion after the introduction of PT. (**D**) 19 October 2018, size of tumour lesions unchanged in comparison to the previous scan.*

The Karnofsky Performance Scale Index was estimated at 60. In mid-December 2017, the patient was hospitalized again for the evacuation of the brain abscess.

From 29 January 2018 to 19 February 2018, the patient was hospitalized at the radiology ward where 3D conformal radiotherapy was performed by using a linear accelerator with power of 6MW. A total therapeutic dose of 40,05 Gy was administered in 15 fractions. During radio and chemotherapy, she continued taking anti-oedema therapy at a dose of 8 mg of dexamethasone per day and, upon completion of RT, the dose was reduced to 4 mg per day. In early March 2018, the patient began to use herbal medicines. FTS combination was introduced immediately. The control scan performed on 20 April 2018 showed a cystic solid tumour process in the temporal region on the right, measuring 78 x 58 x 50 mm **(Figure 5B),** which was accompanied by an extensive oedema. Two satellite nodules could be observed, one in the region of insular lobe with diameter of 15 mm, and another in the posterior temporal region with 13 mm in diameter (**Figure 5B, small arrow**). After this finding, the dose of dexamethasone was increased to 16 mg per day, and the patient continued to take herbal medicines.

In mid-May 2018, following the complete treatment of the brain abscess, the patient started treatment with TMZ at a total daily dose of 200 mg/m^2 of body surface area per Stupp protocol. She continued to use herbal medicines in combination with TZM.

The control MRI imagining, performed on 30 July 2018, a large residual multicentric process could still be observed in the temporal region on the right, but it was significantly smaller in size. At this scan, a larger, symptomatic tumour was measuring 54 x 50 x 30 mm (**Figure 5C**). The satellite nodules were also smaller in size, measuring 11 mm of the nodule in the region of insular lobe, and 10 mm of the nodule in the posterior region of the temporal lobe (**Figure 5C, small arrow**). Concurrently with the regression of the tumour, there was a reduction of the zone of cerebral oedema, so the daily dose of dexamethasone was reduced to 2 x 6 mg per day. In early October 2018, the patient completed the 6th cycle of treatment with TMZ, i.e. she completed the oncological treatment. The dose of dexamethasone was reduced to 2 × 4.5 mg per day. The control scan performed on 19 October 2018 showed that there was still a large residual process located in the frontal-temporal-parietal region on the right which, in comparison with the scan from 30 July 2018, remain unchanged. In addition, the scan showed a stationary size and a somewhat stronger opacification of satellite nodules located in the anterior subcortical region of the insular lobe and in the posterior region of the temporal lobe **(Figure 5D)**. After this finding, the patient continued to take the same combination of herbal medicines.

Discussion

There is no clear protocol for treating multiple GBMs. The main dilemma is whether these patients should undergo a surgical procedure as a part of oncological treatment. On one side, there are authors who advocate the surgical removal of the larger (symptomatic) tumour, followed by treatment with radio and chemotherapy (RT/CT) [9]. Another group consists of those who consider surgery to be too risky and hold that it only slightly prolongs survival, which is why they suggest a treatment with the combined RT/CT after biopsy is performed [**3**]. In patients with multiple GBMs physicians rarely opt for surgery, so the lack of therapy options is certainly one of the reasons why modest results are achieved in their treatment [2].

This paper presented the results of using phytotherapy (**PT**) in 5 patients diagnosed with multiple GBMs. The patients used PT in combination with oncological treatment, and continued to use it after the completion of medical treatment. In the first two patients, there was a primary GBM and the planned oncological treatment was not fully completed. In the first patient, a standard GBM treatment protocol was planned in accordance with Stupp protocol, including the surgical removal of the symptomatic tumour [10]. He applied for PT after completing the combined radio and chemotherapy (RT/CT), when the standard PT (StPT) was introduced.

However, after three cycles of monotherapy with TMZ and PT, a progression of both tumour lesions was performed, and the oncological treatment was discontinued. The patient continued the treatment only with herbal medicines, and instead of StPT, the PTS combination of herbal medicines was introduced. Over the next 15 months, the control scans showed a halt in the growth of the symptomatic tumour **(Figures 1E and 1G)** and continuous regression of secondary lesions **(Figures 1F, 1H and 1J)**. Since at the time this patient was only treated with PT, we can safely conclude that such a result is due to the action of pharmacologically active substances from herbal medicines.

Phytotherapy of salvation (PTS) as the first choice of treatment

In the second patient, the first scan performed on 30 October 2014showed a larger tumour was measuring 29 x 26 x 27 mm (**Figure 2A**). After that, a sterotactic biopsy was performed, and another scan performed 12 days later showed that the tumour measured 43 x 37 x 41 mm (**Figure 2B**). Often after a biopsy, there is rapid tumour growth in patients who are suffering from GBM. Studies regarding this issue have shown that obtaining the tumour tissue samples causes a change in the behaviour of tumour cells and leads to their migration and proliferation [11]. It is quite certain that, in this particular case, the biopsy also led to the progression of tumour growth.

In this patient, it was found that a promoter methylation of *MGMT gene* was not detected, which classified him into the group of patients who did not benefit from the treatment with temozolomide (TMZ) or such benefit is negligible [12]. However, physicians decided to treat the patient by the Stupp protocol, which included the introduction of TMZ, first in combination with RT, after which another 6 cycles of monotherapy with TZM alone were planned [10].

PT was introduced in mid-December 2014, along with the combined RT/CT. Experience with the previous patient, when there was a halt in tumour growth after the introduction of the combination of herbal medicines marked as PTS, as well as the accelerated progression that occurred after the biopsy, were the reasons why PTS was instantly introduced into the treatment of this patient. Since after 33 days of the treatment with the combined RT/CT, the patient developed severe

thrombocytopenia, the treatment was continued only with RT and PT. As the oncological treatment of this patient was practically reduced to radiotherapy alone, further tumour progression and a short survival period were expected. The control scan performed 5.5 months later showed a moderate increase in the larger tumour lesion, measuring 53 x 35 x 54 mm (**Figure 2C, white arrow**).

It should be noted that the treatment of this patient started in mid-December 2014, i.e. one month after the MRI imaging found tumour progression which had occurred after taking the tissue sample for analysis. It is quite certain that the tumour continued to grow until the commencement of treatment; an increase in the dose of corticosteroids during that period indicates so [10]. Therefore, it was not possible to estimate the extent to which the applied treatment had helped the patient, as the tumour dimensions were unknown at the beginning of the treatment. In order for more reliable conclusions on the results of the treatment to be drawn, the next scan had to be performed. It was, however, quite certain that the therapy carried out had benefited the patient, which was indicated by a reduction of the secondary lesion from 22 to 8 mm (**Figure 2C, black arrow**).

This is the only patient out of five who was at the same time treated with radiotherapy and herbal medicines. Since there are reports that artemisinin and its derivatives increase the sensitivity of the glioblastoma cells to radiotherapy, it is certain that this joint activity of RT and PT has led to a significant slowing in tumour growth [13].

Over the next 6 months, the general condition of the patient had significantly improved, and the effectiveness of the combination of PTS was confirmed in July 2015 when the control scan showed a tumour regression (**Figure 2D**). After this six-month period of stability, the patient started frequently feeling weakness in his lower limbs and difficulties walking. The control scans performed in November and December 2015 showed a moderate progression of the primary tumour, and later of the secondary tumour (**Figures 2E and 2F**). The further moderate but steady growth of both tumour lesions (**Figures 2G and 2H**) led to ever-growing difficulties in walking, accompanied by painful and swollen limbs, due to which the patient had to spend an increasing amount of time in bed. In about 2-3% of patients with brain tumours, as a result of a motor deficiency, there is a creation and propagation of the thrombus into the blood vessels of the lungs, which often has a fatal outcome [14]. In mid-July 2016, due to tachycardia and chest pain, the patient was urgently transferred to a hospital where he died shortly after due to a pulmonary embolism. Bearing in mind that that the main and practically only oncological treatment that this patient underwent was radiotherapy, such a long survival period would certainly have not been possible without the use of herbal medicines.

In the third and the fourth patient, the PTS combination of herbal medicines was introduced after the malignant transformation of the previously treated diffuse astrocytoma. Currently, there is no clear strategy for the treatment of diffuse astrocytoma. After recent studies showed that surgical removal of the tumour in the dormancy stage does not affect the survival of the patients, physicians are increasingly opting for monitoring and more frequent controls, and start the therapy only after the tumour begins to grow or after it progresses to a higher grade [15].

In the third patient, after performing a biopsy and diagnosing diffuse astrocytoma, the physicians opted for the treatment because, due to its infiltrative growth and pressure on the surrounding structures, the tumour was seriously compromising the health and the life of the patient. Due to the tumour position and its multifocal spread, the surgery could not be performed and the patient was treated with radiotherapy. A follow-up control check 3 months after the radiotherapy showed a tumour progression to a higher grade (**Figure 3B**).

After the progression, the oncological treatment was continued with TMZ in 6 cycles of monotherapy with TMZ by Stupp protocol. Along with TMZ, the PTS combination of herbal medicines was introduced. The first scan after the introduction of this combined therapy showed a tumour regression **(Figure 3C)** which could be attributed to the joint action of PT and TMZ. However, further tumour reduction and a regression of brain oedema, when the only treatment was PT, could have occurred only due to pharmacologically active ingredients from herbal medicines (**Figures 3D, 3E and 3F**).

In the 4th patient, the physicians detected a tumour lesion by MRI imagining that corresponded to diffuse astrocytoma by its morphological characteristics, and opted for monitoring without introducing the therapy (**Figure 4A**). Two years later, due to a tumour growth, a biopsy was performed, which confirmed the diagnosis of diffuse astrocytoma (**Figure 4B**). The control scan performed 6 months after the biopsy recorded a further tumour growth (**Figure 4C**).

Due to continued tumour growth after the biopsy, radiotherapy was introduced to the patient, and the control scan performed after the completion of the RT showed further tumour growth (**Figure 4D**). In addition, several newly formed smaller zones of imbibition of the contrast were observed in the temporal region on the left and suspected in the temporal region on the right. The finding indicated a tumour progression to a higher grade, which was confirmed by examining the tumour tissue sample obtained after the surgery. Since a further tumour progression was observed by the control scans performed 2 months after the surgery (**Figures 4E, 4F and 4G**), the prognosis was very poor and a short survival period was expected. In this case, oncological treatment was also continued with TMZ by Stupp protocol, and along with TMZ the patient had started using the PTS combination of herbal medicines. After the introduction of TMZ/PT therapy, control scans showed continuous tumour regression (**Figure 4H**). The patient received a total of 12 doses of TMZ, and then continued using only herbal medicines until there were no radiological signs of tumour on 2 consecutive control scans.

In both patients with diffuse astrocytoma, tumour progression occurred after the biopsy was performed. Several studies have shown that biopsy in patients with diffuse astrocytoma reduces their survival rate [15]. It is not excluded that the biopsy in these 2 patients could have changed the course of the disease and accelerated the progression.

The fifth patient was probably the most severe case of all the presented cases. Due to the massive tumour at the time of diagnosis (**Figure 5A**), a large cerebral oedema and his poor general condition, a short survival period was expected [7]. The presence of the brain abscess had also contributed to the poor prognosis, which was the reason TZM could not be included along with the radiotherapy [16]. Contrary to expectations, after the introduction of PTS combination of the herbal medicines, and somewhat later also TMZ, the patient's general health improved considerably. The improvement at first manifested as a reduced need for corticosteroids. The final confirmation of the effectiveness of the applied treatment was after control NMRI imaging 5 months after the introduction of PT, when regression of tumour lesions was observed (**Figure 5C**). Additional evidence of the effectiveness of the therapy appeared in October 2018 when the control scan showed no progression of the disease (**Figure 5D**).

After the authors of this study, in their previous paper, demonstrated that phytotherapy had its place in the treatment of patients suffering from solitary GBM (Trogrlic et al., 2018), the results presented in this paper support their use in multiple GBMs [**7**]. On this occasion, it has again been shown that PT in combination with TMZ gives excellent results, especially in patients with the progression of diffuse astrocytoma to a higher grade. It should be emphasized that, in the 4th patient, there was a complete regression of tumour residue and all secondary lesions due to combined activity of TMZ and herbal medicines.

It is important to point out the potential of the herbal combination marked as PTS in treating patients suffering from GBM, as well as to suggest that it should be the first choice of treatment for all patients in the future. In this combination of herbal medicines, the content of plants from the genus *Artemisia* L. is twice as high as that found in StPT. This requires more frequent control of liver markers, as studies on the hepatotoxicity of artemisinin derivatives in combination with TMZ showed that this combination could lead to liver damage [17]. However, laboratory analyses have never shown any significant growth of such markers. The patients used PT for a period ranging from 8 to 33 months, and no side effects were observed in any of them. Our study has shown that oncological treatment and treatment with herbal medicines are not mutually exclusive, which opens the possibility of the simultaneous use of these two methods of treatment.

The recommendation to the subjects was to use PT as long as there were clinical and radiological signs of tumour. For this reason, in the 4th patient, PT was discontinued 33 months after its introduction, after 2 consecutive control scans showed no signs of tumour, while patients 3 and 5, at the time of the publication of the results of this study, were still using herbal medicines.

Conclusion

New brain imaging techniques that allow for the more precise and reliable detection of multiple lesions have shown that, in more than 30% of patients, glioblastoma presents at two or more locations. It is certain that this percentage, will continue to grow with the development of new imaging techniques, for which modern medicine is completely unprepared. The modest results achieved today in the treatment of these tumours are the result of the limited number of therapy options, and it is necessary to introduce new methods of treatment. The results presented in this paper open the possibility of introducing phytotherapy, which would contribute to the more effective treatment of patients with multiple glioblastoma in combination with the standard oncologic treatment.

Declarations

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Ethics declarations

Ethics approval and consent to participate: Collection of specimens used in this work was done according to regional and national guidelines. No permission was requested as the plants speciments used in this study is not under any protective figure.

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