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# Effect of Zinc Sulfate on Overall Survival and Progression-Free Survival in Patients with Glioblastoma Multiforme, a Phase II Study

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## Abstract

**Background:** Glioblastoma is the most prevalent and aggressive adult glial tumor. Patients who receive standard treatment have a mean survival of 12-14 months. Zinc is a micronutrient that has shown to have anticancerous effects. In the in vitro studies zinc had antineoplastic effects on gliobalstoma cells.

**Method:** This is a phase II randomized trial in which 60 patients in two groups were evaluated. The zinc group (29 patients) received zinc sulfate supplement 50 mg orally twice a day and the control group (31 patients) who were selected from historical case sreceived no supplements.

**Results:** Mean overall survival in the case and control groups were 9.93 (± 3.29) and 9.0 (± 3.56) months. In the case and control groups, the mean disease-free survival were 9.62 (SD ± 3.37) and 8.26 (SD ± 3.47) months. These differences were not statistically significant. Although overall survival and recurrence-free survival in patients in the case group was higher than the control group, there was no statistically significant difference ($P = 0.485$).

**Conclusion:** Zinc consumption was associated with better survival, but these differences were not statistically significant, necessitating further studies.

***Keywords:*** Glioblastoma multiforme, Chemotherapy, Radiotherapy, Zinc sulfate

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## Introduction

Glioblastoma is the most widespread and aggressive adult glial tumor.[1] It is more common in men and may occur at any age, but its prevalence increases with age. The peak of prevalence is from 45 to 70 years of age.[2] If no treatment is done,

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patients with glioblastoma would die in 3 months. Patients receiving standard treatment have an average overall survival (OS) of 12-14 months, with less than 25% living up to 2 years and less than 10% up to 5 years.[2-5]

Zinc is a micronutrient andan essential component of certain important enzymes.[6-7] In an in vitro study, zinc increased apoptosis when added to glioblastoma cells.[8] In another study, addition of ZnCl2 to alkylating drugs in tumor cells strongly increased the sensitivity of tumor cells with P53 mutation to antitumor drugs.[9]

The recommended daily dose of zinc is 11 mg for men and 8 mg for women.[11] These are far from the lethal dose of 50% (LD50), which is equivalent to 27 gram per day, and have been obtained by comparison with similar studies in rats and mice.[11] Consumption of such a large amount of zinc in humans is intolerable because a dose of about 225-400 mg causes vomiting.[12] Although severe zinc deficiency is rare, mild zinc deficiency is prevalent even in developed countries.[13] The zinc deficiency prevalence is more than 20% around the world.[14]

In this study, we aimed to investigate the effect of zinc supplementation on the survival of patients with glioblastoma.

## Materials and Methods

This study was a case-controlled phase II study on patients with glioblastoma in the radio-oncology department of Nemazee Hospital, Shiraz University of Medical Sciences (2019-2020).

All patients were newly diagnosed glioblastoma who had undergone surgery and were aged between 18 and 70 years; they had ECOG <2, normal blood cell count, liver and kidney function tests, and no history of other cancers or systemic diseases. Exclusion criteria were history of previous brain tumor, allergy to zinc or temozolamide, consumption of zinc supplementation during the last two weeks (considering that the half-life of zinc in the body is 11 days) and patients' refusal to continue.

All patients signed a written informed consent. Demographic and clinical characteristics of patients such as age, sex, past medical history, and physical examination were recorded. Serum zinc levels were measured prior to the treatment. In addition, due to the effect of zinc on the reduction of serum copper, the serum level of copper was measured as a baseline to ensure that it was not deficient. Zinc level was further measured twice, during treatment and after treatment, to ensure no disturbances during the study.

Patients with glioblastoma, after maximal safe tumor resection, were referred to our department. They received external beam radiotherapy (EBRT) up to 60 Gy with three-dimensional conformal radiotherapy technique and concurrent chemotherapy with temozolamide at a dose of 75-100 mg/m$^2$/day. Following radiotherapy, adjuvant chemotherapy was administered to all patients at a dose of 150-200 mg/m$^2$/day; this continued for 5 days, every 28 days for 6 cycles.

In the case group, patients took 2 capsules (50 mg in each) daily with at least 2 hours before and after temozolamide. Zinc was started with radiotherapy and continued until the end of treatment (with the final cycle of chemotherapy). Two weeks before the treatment, patients were asked to stop taking any vitamins and supplements.

Control group (31 people) consisted of patients who did not receive zinc sulfate and were chosen retrospectively from patients with glioblastoma based on inclusion and exclusion criteria.

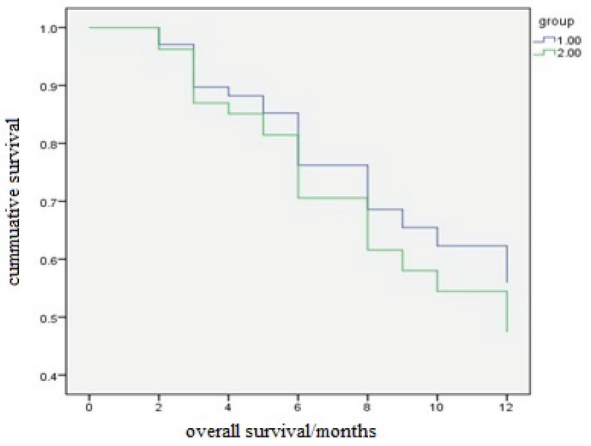


**Figure 1.** This figure shows overall survival in patients with glioblastoma who received (group 1) and did not receive (group 2) zinc.

**Table 1.** Demographic and clinical characteristics of patients with glioblastoma multiforme between case and control groups

| Characteristics | Case (n = 29) | Control (n = 31) | *P* – value |
|---|---|---|---|
| **Age, mean ± SD** | 50.96 ± 13.6 | 55.3 ± 11.02 | 0.72 |
| **Sex (male), n (%)** | 19 (65.5) | 18 (58.06) | 0.20 |
| **Sex (female), n (%)** | 10 (34.5 ) | 13 (41.9 ) | 0.20 |
| **Left side location, n (%)** | 12 (41) | 17 (55) | 0.36 |
| **Right side location, n (%)** | 17 (59 ) | 14 (45) | 0.36 |
| **Tumor size (cm)** | 7.3 (± 2.9) | 7.4 (± 3.1) | 0.89 |
| **Performance Status** | 29 | 31 | 0.13 |
| **Size of remnant tumor(mean/mm)** | 41.1 | 30.05 | 0.37 |
| **Radiotherapy doses(Gy)** | 58.3 | 55.7 | 0.59 |
| **Chemotherapy cycles** | 5.19 | 5.3 | 0.82 |

SD: Standard deviation

Patients were followed up weekly for complete blood count, acute toxicity, and treatment protocol adherence. Reported toxicities were nausea, diarrhea, heartburn, abdominal pain, weight gain, headache, and anemia, all recorded in the data collection form. None of these side-effects caused treatment interruption or change. It is noteworthy that not all of these side-effects are related to zinc and could be related to temozolomide.

Zinc and copper serum levels were measured in the third month after the start of the treatment and at the end of the treatment (seventh month). Consuming zinc for more than 10 weeks interfered with the absorption of copper in the body; serum copper levels were measured three months after taking zinc to find cases with copper deficiency and determine the necessary course of action.

In this study, OS was defined as the period from the first day of diagnosis to death, and disease-free survival (DFS) was defined as the period from the first day of diagnosis to disease recurrence or progression.

The Ethics Committee of Research inShiraz University of Medical Sciences (IR.SUMS.MED.REC.1398.071) approved this study. This study was registered in the National System of Clinical Trials (IRCT201904300432N1) and the patients were assured that it would not be harmful. In addition, all the information obtained from this study was kept confidential by the researcher and the patients had the right to leave the study at any time.

## Results

A total of 60 patients with primary glioblastoma entered the study (Table 1) and the mean ages of the cases and controls were 50.96 ±13.6 years (median 0.57 and range 18-70) and 55.3 ± 11.02 years (median 0.60 and range 20-70), respectively. The difference was not statistically significant. The two groups were age-matched ($P = 0.720$). In the case group, 19 (65.5%) were males and 10 (34.5%) were females, while in the control group, 18 (58.06%) were males and 13 (41.9%) were females. There was no statistically significant difference between the two groups in terms of gender ($P = 0.204$)

The two groups were compared for tumor residue after surgery. All patients underwent maximal safe surgery with a mean residual size of 41.1 (± 30.54) in the case group and 30.5 (±30.55) mm in the control group, which was statistically similar ($P = 0.372$). Mean serum zinc levels were 85.88 micrograms per deciliter before the treatment and 93.6 micrograms per deciliter after the treatment.

In the case group, 65.5% of the patients and in the control group, 69% received a full course of treatment. In other words, 34.5% and 31% of each group received incomplete treatment. The cause of treatment interruption in all patients was death or worsened general condition. In the zinc group, 1 patient died after receiving 40 Gy radiotherapy, and 9 patients were not able to finish chemotherapy. In the control group, 2 patients died after receiving 31 and 28 Gy radiotherapy and 7 patients were not able to finish chemotherapy. This difference was statistically comparable and without significance ($P = 0.599$). The mean dose of radiotherapy was 58.3 (± 3.07)

Gy in the case group and 55.7 (± 3.25) Gy in the control group, which does not show any statistical significance ($P = 0.484$). The number of adjuvant chemotherapy cycles was 5.19 cycles (SD ± 1.55) in the cases and 5.3 cycles (SD: ± 1.49) in the controls, but there was no significant difference ($P = 0.820$).

In control and case groups, 13 and 11 patients passed away, while 18 and 18 patients survived. The mean OS in the case group was 9.93 (SD ± 3.29) months and its range was 3-12 months. In the control group, the mean OS was 9.0 (SD ± 3.56) months and its range was 2-12 months. In the case group, the mean DFS was 9.62 (SD ± 3.37) and in the control group, it was 8.26 (SD ± 3.47).

One-year OS in the cases was 55.2%, which is higher than the controls (48.4%), but this difference was not statistically significant (Figure 1).

## Discussion

In this study, the patients were divided into case and control groups. The case group (29 patients) received zinc sulfate supplement 50 mg orally twice daily and in the control group (31 people), zinc was administered, and they were selected from those patients who were treated at our department. The OS was 9.93 (SD ± 3.29) months in the case group and 9 (SD ± 3.56) in the control group. Although OS in the case group was higher than the controls, there was no statistically significant difference. DFS was 9.62 months in the case group and 8.26 months in the control group.

Glioblastoma is the most prevalent and aggressive glial tumor.[1] It may occur at any age, but its prevalence increases with age.[2] The mean interval to recurrence following standard treatment is 6.9 months.[15]

46%-70% of zinc is absorbed from bowel. It is commulated in brain and has different concentrations in different parts of brain. The mean concentration of zinc is 10 times more than that of plasma.[16] Some studies have shown the importance of zinc as an antioxidant or free radical scavenger.[6, 17] However, its role in cancer prevention has not been proven. In a study, Franklin et al. showed that high levels of zinc in prostate cells, through metabolic and intracellular signaling pathways, had antiproliferation and apoptosis effects. Zinc deficiency may cause this imbalance and progression to cancer.[18]

In 1998, in a study by Timar et al. on mice with liver metastasis, high doses of zinc reduced the number of liver metastases, which had no effect on lesion size and survival in mice.[19] In a study by Kocdor et al., cells with non-small cell lung carcinoma underwent chemotherapy with zinc supplementation and showed that zinc enhances the effect of docettaxel, increases apoptosis, and inhibits the growth of malignant cells. Effects of zinc were observed in both P53 mutant and wild type cell lines.[20]

Toren studied human glioblastoma cells in four groups: the first cell culture medium received no treatment; the second group received DMSO (solvent of TMZ) + ZnCl2; the third cell culture medium received only temozolamide, and the last group received TMZ + ZnCl2 combination. Each group of human cell culture media consisted of three subsets: a culture medium with glioblastoma cells with P53 mutation and a culture medium with glioblastoma cells P53–Wild type and a culture medium with normal astrocyte cells in each of the four arms. In their study, the addition of TMZ + ZnCl2 to Glioblastoma culture medium with and without P53 mutation significantly reduced the number of cells due to increased apoptosis and decreased proliferation in the remaining cells. In addition, zinc was able toactivate the P53 gene in the P53 –wild type group and subsequently enhance the effects of chemotherapy.[21]

Puca and Nardinocchi investigated the effect of adding ZnCl2 to alkylating drugs in tumor cells with P53 mutation in breast and glioblastoma cell line. The culture medium of glioblastoma cells was treated with cisplatin alone in comparison with the combination of cisplatin and ZnCl2. Tumor cell death was significantly higher in those who received both cisplatin and ZnCl2. They concluded that zinc supplementation strongly increases the sensitivity of tumor cells

with the P53 mutation to antitumor drugs.[9]

In a cohort study by Epstein et al. in Sweden, zinc (more than 14 mg daily) was associated with a reduction in prostate cancer mortality in patients with prostate cancer, and this association was more pronounced in localized diseases.[22] In 2009, Yung-Song Lin studied 34 patients with advanced nasopharyngeal cancer, administering 75 mg of zinc daily for two months, while standard treatment was done. This reduced local recurrence and improved patients' OS compared with placebo, but metastasis was not reduced.[23]

The main limitation of this study was small size of patients. In a properly large study the reported difference may be more obvious.

## Conclusion

The results of this study showed that in patients with glioblastoma multiforme undergoing chemoradiotherapy, zinc supplementation increased OS and DFS but had no significant effect. Zinc sulfate supplementation had acceptable safety in the studied patients and no serious side-effects were reported following its use. Based on the evidence in previous studies on the anticancer effects of zinc supplementation, further studies in the future are necessary to confirm or disprove the results of the current research.

## Conflict of Interest

None declared.

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