Frequency of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* in Patients Suffering From Glioma Compared to Healthy Individuals

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**Abstract**

**Background:** Glioma is a type of the primary tumors that progresses in the spinal cord and brain. Probiotics have probably a protective impact against the disease, especially when treated as a microbial flora in the gut.

**Objectives:** Given the effective role of microbiota in body function, this study aimed to investigate the frequency of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* in patients suffering from glioma compared to healthy subjects.

**Materials and Methods:** To achieve our study objectives, 20 patients with glioma tumors as well as 20 healthy individuals were enrolled. Stool samples were taken from the subjects and stored at minus 20 degrees after processing. Real-Time PCR technique was employed to assess the change in the copy number of *L. acidophilus*, *L. plantarum*, and *E. faecalis* in patients with glioma in comparison to healthy individuals.

**Results:** The results showed significant difference between the population of *L. plantarum* in the patients and healthy individuals (*P* = 0.0004). The patients with glioma were in the 35-60 age range and the mean age of healthy individuals was 45 years (*P* = 0.48).

**Conclusion:** It was concluded that *L. plantarum* had potential for tumor induction.

**Keywords:** Lactobacillus acidophilus, Lactobacillus plantarum, Enterococcus faecalis, Glioma, Real-time PCR

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**Background**

Brain tumors are among the deadliest diseases that cause thousands of deaths each year.¹ Glioma covers about one-third of all central nervous system (CNS) tumors as well as 80% of malignant brain tumors. The incidence of glioma in men is higher than that in women. Family history of brain tumors accounts for a small percentage of developing tumors. Exposure to ionizing radiation increases the risk of a brain tumor.² The association of colonized microbiota in the gastrointestinal tract with extra-gastrointestinal and gastrointestinal diseases have been reported.³ Moreover, the role of colonized microbiota in the progression of neurologic diseases, and the bilateral relationship between CNS and the intestine known as “microbiota-gut-brain axis” have been also identified.⁴ Probiotics, which are gut microbial flora, have probably a protective impact against the disease especially when they are treated as a microbial flora in the gut.⁵ Among bacteria with probiotic virtues, the *Lactobacillus* family has an outstanding position. *Lactobacillus* and bifidobacteria can be considered as the most popular probiotics in the gastrointestinal tract, which play critical roles in the treatment of gastrointestinal diseases.⁶ To the best of our knowledge, no study has been conducted in Iran to examine the role of probiotics in inducing or preventing glioma. This study, therefore, aimed to
evaluate the frequency of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* populations in patients with glioma brain tumor in comparison to healthy controls in order to identify the potential preventive effects of these bacteria on glioma or their progression.

**Materials and Methods**

**Sampling**
Diagnostic and sampling procedures were performed during three months in 2020 and in Shohada Tajrish hospital, Tehran, Iran. The obtained samples were processed and subjected to quantitative absolute real-time polymerase chain reaction (PCR) in Alborz University of Medical Sciences. A total of 40 samples including 20 individuals with glioma brain tumor (12 males and 8 females) and 20 healthy subjects (10 males and 10 females) were included in this study. Individuals with glioma brain tumor were diagnosed by a neurologist using clinical examination and CT scan. Moreover, cases with glioma positive were confirmed by biopsy and pathological test. The patients receiving antibiotics two months prior to surgery or undergoing chemotherapy as well as those with family history of colon cancer, Irritable bowel syndrome (IBS) or Inflammatory bowel disease (IBD), and regular consumption of non-steroidal probiotics were excluded from this study. The samples were transferred on ice and then stored at minus 20 degrees after pre-processing.

**Preparation of Standard Strain**
Standard DNA of *Lactobacillus plantarum* (DSM20174), *Lactobacillus acidophilus* (DSM20079), and *Enterococcus faecalis* (ATCC: 29212) were obtained from the Iranian Biological Resource Center (IBRC).

**Genomic DNA Extraction**
The extraction of genomic DNA of samples were carried out by utilizing Qiaamp DNA stool mini kit (Qiagen). The concentrations of DNA were specified with the Nano Drop 2000 (Thermo. Scientific). Integrity size of DNA were tested by 1.5% agarose gel electrophoresis and then stored at −20°C before amplification stage.

**Polymerase Chain Reaction**
Forward and reverse primers for *L. acidophilus*, *L. plantarum* and *E. faecalis* were 5′-AATCTCTTCTCGGTCGCTCTA-3′; 5′-CCTTTCTAAGGAAGCGAAGGAT-3′; 5′-CACGGTTGCGATTACGGTTGTTCC-3′, and 5′-GCCGCCTAAGGTGGGACAGAT-3′; 5′-TTCCTCTAAGCGAAGGAT-3′; 5′-CGAAAGATCCAGCAGATGCGGTT-3′; 5′-GCCGCCTAAGGTGGGACAGAT-3′; and 5′-ACGGTGCTCGGATACGGTTGTTCC-3′, respectively. The specificity was confirmed by PCR in 50 μL reaction mixtures containing 1 μL (each) primer, 3 μL DNA-template, 25 μL PCR master mix, and 20 μL Deionized Water (DW). PCR was performed with denaturation step of 95°C for 45 seconds, annealing stage of 55°C for 40 seconds, and the extension stage of 72°C for 25 seconds followed by 45 cycles. The visualization of DNA bands was performed by UV illumination to confirm the production of 166-bp (*L. plantarum*), 176-bp (*L. acidophilus*), and 126-bp (*E. faecalis*) amplicons. To ensure specific primer binding, the PCR product was sequenced and the result was confirmed by BLAST.

**Real-time Quantification PCR of Total Lactobacillus plantarum, Lactobacillus acidophilus, and Enterococcus faecalis Load**
Quantitative RT-PCRs were carried out in 20 μL reaction volume containing 10 μL SYBR Green PCR Master Mix (Takara, Otsu, Japan), 0.5 μL of each forward and reverse primers, 2 μL of DNA extracted from the fecal samples, and 7 μL DW. The reaction conditions were initial denaturation 94°C for 2 minutes, followed by 35 cycles of 94°C for 20 s and 55°C for 25 s. Purified genomic DNA in the range 1 ng of *L. plantarum*, *L. acidophilus*, and *E. faecalis* were employed as the standard for specification their DNA amount by real-time PCR. The five standard curves real-time PCR was carried out on the cDNA samples using a Q-6000 machine (Qiagen, Germany). The copy number of the bacteria per gram of feces was specified in each specimen according to the five standard curves.

**Statistical Analysis**
Data were analyzed with GraphPad Prism 8 using nonparametric ANOVA test. *P* values of <0.05 were considered as significant.

**Results**

**Demographic Analysis**
The age range of glioma brain tumor incidence was 30-60 years. No significant differences were observed in the patient’s age and sex compared to those of the healthy individuals (*P* = 0.48, *P* = 0.1).

**Real-time Quantification PCR of Total Lactobacillus plantarum, Lactobacillus Acidophilus, and Enterococcus faecalis Load**
The results showed that there was a statistically significant difference between the population of *L. plantarum* in glioma patients and healthy individuals (*P* = 0.0004). However, no statistically significant differences were observed between *L. acidophilus* (*P* = 0.82) and *E. faecalis* (*P* = 0.71) populations. Copy number information of *L. plantarum*, *L. acidophilus*, and *E. faecalis* in the evaluated groups are shown in Table 1.

**Discussion**
In this study, individuals with glioma brain tumor and healthy control subjects were examined to determine the
frequency of *L. plantarum*, *L. acidophilus* and *E. faecalis* among the populations. The results revealed significant differences regarding *L. plantarum* population, but no meaningful differences were found concerning *L. acidophilus* and *E. faecalis* populations in the given groups. These results, for the first time, demonstrated that the population of *L. plantarum* was significantly larger in subjects with glioma compared to that in normal individuals. Our result was inconsistent with the results from other studies showing a lactobacilli population decrease in cancers. *L. plantarum* have the potential to be introduced as probiotics. Despite the numerous beneficial effects, there are sometimes restrictions on its use since, for example, some breeds cause food spoilage or even sometimes become pathogenic. These probiotics can influence the cancer or tumor growth rate by inducing apoptosis or by causing diseases such as intestinal inflammation and diseases of the nervous system such as migraine and multiple sclerosis.9-10

*Lactobacillus acidophilus* is a major probiotic bacterium and a lactic acid one in human colon. Extensive research has been conducted to investigate the changes of this bacterium in various diseases as well as its probiotic beneficial effects and other *Lactobacillus* species. Zinatizadeh et al, for instance, found that the number of Lactobacillus acidophilus colonies in colorectal and polyp patients was decreased compared to that in healthy individuals and, therefore, suggested that the population of this organism was associated with polyp and colorectal cancer.11 Several studies have also examined the role of this bacterium in controlling colorectal cancer.12-15 *E. faecalis* has been reported to correlate with colorectal cancer.16 In this study, however, no remarkable difference was detected between patients and healthy individuals regarding *E. faecalis* population.

**Conclusion**

In conclusion, the role of *L. plantarum* in the induction of glioma was not definitely determined; however, our study results may have been used as an incentive for conducting further focused researches in the fields of epidemiology, diagnostic, therapeutic or clinical interventions. Further researches with more exclusive and larger sample sizes than that of this study may have generated more robust results for the scientific community.

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**Authors’ Contribution**

EA: data analysis, laboratory experiments, and writing of the manuscript. SY: laboratory experiments, data analysis. PF: laboratory experiments and data analysis, S O: clinical diagnosis and sampling, H J: clinical diagnosis and sampling, AV: samples providing. KK: data analysis. AB: study design.

**Conflict of Interest Disclosures**

The authors declare that they have no competing interests.

**Ethical Approval**

This study was approved by Alborz University of Medical Sciences, Karaj, Iran (Ethical code: IR.ABZUMS.REC.1397.116).

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**Table 1.** Copy number information of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* in the evaluated groups

| Abbreviations: LPT, *Lactobacillus plantarum* in glioma patient; LPC, *Lactobacillus plantarum* in normal individuals; LAT, *Lactobacillus acidophilus* in glioma patient; LAC, *Lactobacillus acidophilus* in normal individuals; EFT, *Enterococcus faecalis* in glioma patient; EFC, *Enterococcus faecalis* in normal individuals. Significant differences were specified in the population of *Lactobacillus plantarum* in gliomas compared to healthy individuals. |
| P value | 0.0004 | 0.8251 | 0.7127 |
| Number of values | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Minimum | 1.68 × 10^4 | 0.000 | 0.000 | 0.000 | 8.37 × 10^4 | 8.85 × 10^4 |
| 25% Percentile | 2.68 × 10^4 | 7.8 × 10^4 | 4.43 × 10^2 | 8.1 × 10^3 | 5.88 × 10^4 | 2.87 × 10^4 |
| Median | 6.31 × 10^4 | 9.8 × 10^5 | 8.25 × 10^2 | 1.75 × 10^3 | 3.2 × 10^5 | 1.64 × 10^5 |
| 75% Percentile | 1.76 × 10^7 | 5.48 × 10^6 | 6.08 × 10^3 | 2.33 × 10^3 | 2.47 × 10^6 | 4.71 × 10^6 |
| Maximum | 1.36 × 10^8 | 1.43 × 10^7 | 4.97 × 10^4 | 6.19 × 10^4 | 3.21 × 10^7 | 3.48 × 10^7 |
| Mean | 1.97 × 10^7 | 3.27 × 10^6 | 6.29 × 10^3 | 4.62 × 10^3 | 4.54 × 10^6 | 5.74 × 10^6 |
| Standard deviation | 3.49 × 10^7 | 4.52 × 10^6 | 1.23 × 10^4 | 1.36 × 10^4 | 9.96 × 10^6 | 1.17 × 10^7 |
| Standard error of mean | 7.81 × 10^6 | 1.01 × 10^6 | 2.76 × 10^3 | 3.04 × 10^3 | 2.22 × 10^6 | 2.63 × 10^6 |
| Lower 95% CI | 3.4 × 10^6 | 1.15 × 10^6 | 4.97 × 10^2 | -1.74 × 10^3 | -1.23 × 10^5 | 2.19 × 10^5 |
| Upper 95% CI | 3.61 × 10^7 | 5.39 × 10^6 | 1.20 × 10^4 | 1.09 × 10^4 | 9.20 × 10^6 | 1.12 × 10^7 |
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