

Dariush Moslemi (MD)¹
Sahar Latifi (DDS, MS)²
Mohammad Mehdizadeh (DDS, MS)^{3*}

1. Department of Radiation Oncology, Babol University of Medical Sciences, Babol, Iran
2. Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Babol University of Medical Sciences, Babol, Iran
3. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Qom University of Medical Sciences, Qom, Iran

* Correspondence:

Mohammad Mehdizadeh,
Department of oral and maxillofacial surgery, Faculty of dentistry, Qom university of medical sciences, Qom, Iran

E-mail:

dr.rmohammad@yahoo.com

Tel: +98 1132190056

Received: 16 Feb 2023

Revised: 10 Nov 2023

Accepted: 27 Nov 2023

Published: 19 Oct 2024

Kefir probiotic products on the count of *Candida Albicans* in saliva of chemotherapy patients: A randomized placebo-controlled single-blind study

Abstract

Background: Oral candidiasis is the most common infection of oral mucosa caused by *Candida albicans*. A common predisposing factor for candidiasis is immune system suppression in specific diseases such as AIDS and various cancers. This study aimed to analyze the effect of Kefir probiotic products on the count of *C. Albicans* in the saliva of chemotherapy patients.

Methods: In this single-blinded clinical trial, 50 patients were selected who have signed informed consent forms. Patients aged 20-60 years with colon or breast cancer who received the same chemotherapy regimen were included and those with a history of radiation therapy, underlying diseases, using antibiotics, anti-fungal and GCSF medicines were excluded. Matched patients in test and control groups received 100 ccs Kefir probiotic and mineral water, respectively at a specific daily time for five weeks. Blood and saliva samples were collected in five steps. Data were analyzed using SPSS Version 21 and the significance level was set at $p < 0.05$.

Results: In saliva samples, the count of *C. Albicans* in the test group dropped significantly ($p < 0.05$), while there were no significant differences between test and control groups in blood samples ($p > 0.05$). Comparing the follow-up sessions, in the test groups, WBC and Neutrophil, and in the control groups, WBC count and hematocrit showed significant differences ($p < 0.05$).

Conclusion: Based on the results, using probiotic products daily over a short-term period drops the count of oral *C. Albicans*. Therefore, Kefir probiotic products can be used as an additional treatment for chemotherapy patients.

Keywords: *Candida albicans*, Kefir, Chemotherapy, Probiotic, Saliva.

Citation:

Moslemi D, Latifi S, Mehdizadeh M. Kefir probiotic products on the count of *Candida Albicans* in saliva of chemotherapy patients: A randomized placebo-controlled single-blind study. Caspian J Intern Med 2025; 16(1): 90-95.

Experimental studies have demonstrated that *Lactobacillus* spp. exhibit various anti-*Candida* activities that have a detrimental impact on the virulence characteristics of *C. albicans*. These activities include inhibiting growth, impeding the yeast to hyphae transition, reducing adhesion, and preventing biofilm formation (1, 2). *Candida* is considered normal flora in human genitalia, urinary, and digestive systems (1). The oral cavity of 20-80% of healthy people contains this microorganism (2, 3). Candidiasis is a very common fungal infection in many hospitals. Oral candidiasis is the most common infection of the oral mucosa caused by *Candida* species in many cases. Two common causes of candidiasis are the long-term prescription of antibiotics such as penicillin, and the weakness of patients' immune systems amid AIDS, chemotherapy, leukemia, and other cancers. Risk factors for oral candidiasis include diminished salivary glands activity, foods with high carbohydrate content, smoking, diabetes, and stress (4-7). Additionally, oral candidiasis is a common problem among elderly people. This infection may be lethal to humans if not treated. People who undergo chemotherapy are susceptible to oral candidiasis (8-10).



Probiotic products have been used in the treatment and prophylaxis of many yeast infections (11). Probiotics are living bacteria in microbial foodstuff that have beneficial effects on the host by reducing inflammation. A wide range of probiotic products includes chewing tablets, dairy products such as milk, cheese, ice cream, and regular probiotics such as curd. In addition, a number of probiotics are beneficial to humans through reducing infections, allergy, lactose activity, and blood pressure (12). Recent studies have shown that *C. Albicans* is involved in carcinogenic development (12). Kefir is a dairy product made from fermented kefir grains. Kefir grains are white and resemble cauliflower, which contains a set of bacteria such as lactobacilli, and certain types of yeasts. Kefir contains vitamins B1 and B2, calcium, and folic acid (13). There is also a small percentage of fat and lactose, thus it could be used in many special diets (14). According to the researches, Kefir grains are effective against various types of gram-positive and gram-negative bacteria, and some fungi (13). This study aimed to analyze the effect of probiotic consumption on *C. Albicans* in patients undergoing chemotherapy.

Methods

Trial design and setting: This study was a single-blinded clinical trial by IRCT ID: IRCT: 201506181760N41, conducted at Rajae Hospital, Babolsar, Iran. According to similar studies, 50 volunteers were considered for the study (1, 2). A total of 50 colon and/or breast cancer patients who received chemotherapy regimens between January 2017 and March 2018 were selected for this study. Patients were then divided into treatment groups who received Kefir Probiotic Dough and the control group who received mineral water. Figure 1 summarizes the study design.

Recruitment: All study participants signed a written informed consent prior to enrolment who were allowed to leave the study anytime. Moreover, this study was approved by the Ethics Committee of Babol University of Medical Sciences.

Study participants: The inclusion and exclusion criteria for participation included the following:

Inclusion criteria: (A) 20–60 years of age. (B) Either colon or breast cancer/ (C) Receiving the same chemotherapy regimen

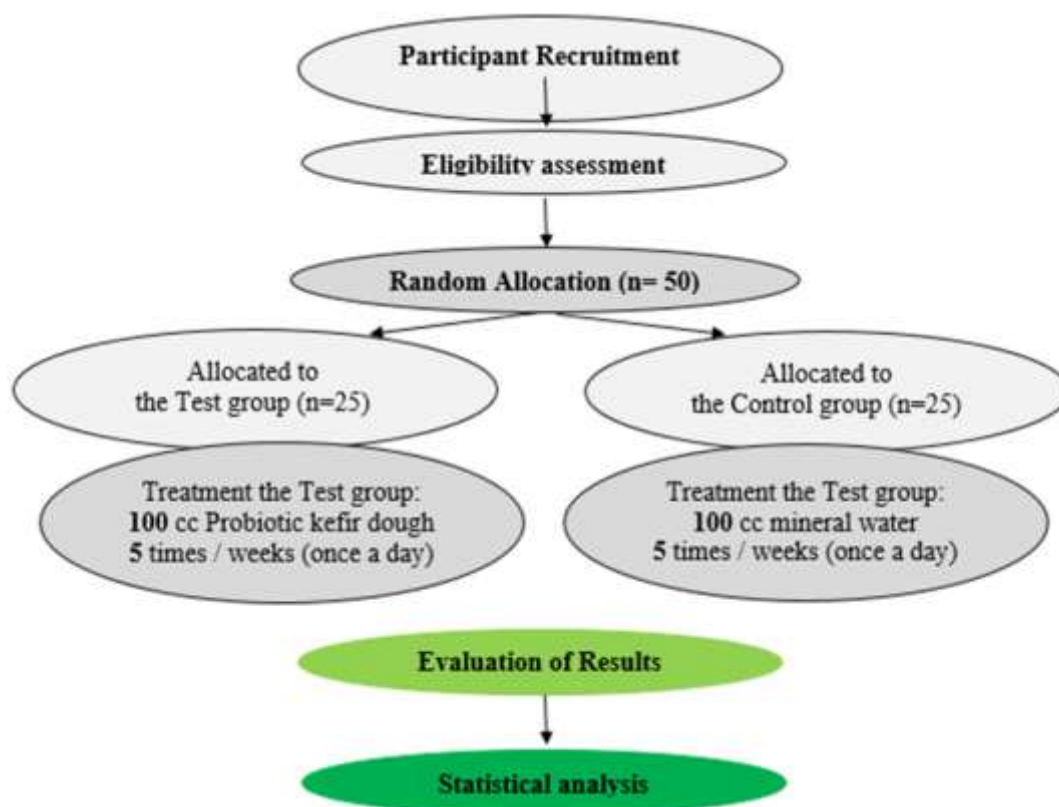


Figure 1. Flowchart describing the study plans

Exclusion criteria: (A) History of chemotherapy, radiotherapy, atrophic tongue, anemia, or underlying diseases such as diabetes and blood pressure. (E) History of mucosal skin. (B) Pregnant women. (C) Smokers. (D) Using antibiotics, antifungal and GCSF medicines.

Intervention: Patients were randomly assigned to control or treatment groups at a 1:1 ratio. Both groups were equivalent regarding the number of patients with colon and breast cancers. Kefir Dough and mineral water were carried in similar opaque containers. Only the person labeling the containers was aware of the solution in each container so that therapist and analyst intervention in treatment results and analysis was prevented. Based on the study guidelines, 100 ccs of the probiotic kefir dough and mineral water were administered to test and control groups, respectively, five times per week at a specific time.

Sampling procedures: In this study, blood and saliva samples were obtained five times. Complete Blood Count (CBC) was carried out on blood samples to determine the count of White Blood Cells (WBC), Red Blood Cells (RBC), Neutrophils (N), Platelets (PLt), and Hematocrit (HCT). In addition, saliva samples were used to determine the count of *C. Albicans* yeast cells present in the normal saliva flora. Each time, 1 cc of saliva sample was obtained before breakfast, transported to the laboratory, and centrifuged. The proposed method for determining the count of *C. Albicans* was the counting method, which takes into account both dead and live cells. 20µm of saliva was

centrifuged and poured on a hemocytometer and the total number of fungal cells was counted. At the next chemotherapy session, saliva and blood samples were obtained before chemotherapy. Afterwards, second, third and fourth samplings were done with one-week interval. Final sampling was carried out at the end of the probiotic consumption period. The culture method was used to count live yeast cells on the culture plate. At the end of the consumption period in both groups, the effect of consuming probiotics was examined regarding the relation between *Candida* development and WBC count.

Statistical analysis: Data were analyzed using SPSS Version 21. The significance level was set at 0.05.

Results

In this study, 50 patients undergoing chemotherapy were evaluated. Patients' demographic information is shown in table 1. As shown in table 2, the count of *C. Albicans* increased significantly in control group ($P = 0.029$) and on the other hand, *C. Albicans* had a significant decrement in test group ($p < 0.001$). Also, the results showed a significant difference in count of *C. Albicans* between two groups ($P < 0.001$). In addition, in blood indices, there were no significant differences between test and control groups ($P > 0.05$). There was a significant difference between the first and the last blood samples regarding WBC in both groups ($p < 0.05$) (figure 2A).

Table 1. The information of the participants in the study

Total Participants= 50			
Test.G = 25		Control.G = 25	
Breast.T	Colon.T	Breast.C	Colon.C
14	11	13	12

Table 2. Studied Indices in Test and Control Groups

Index Change Source	Count of <i>Candida Albicans</i>	WBC	RBC	Hb	HCT	PLt	N
Between-Subjects	<0.001 *	0.838	0.497	0.676	0.255	0.987	0.950
Test Group (Within-Subjects)	<0.001 *	0.042 *	0.823	0.352	0.093	0.062	0.01 *
Control Group (Within-Subjects)	0.029 *	0.04 *	0.723	0.684	0.037 *	0.192	0.172

(*: significant)

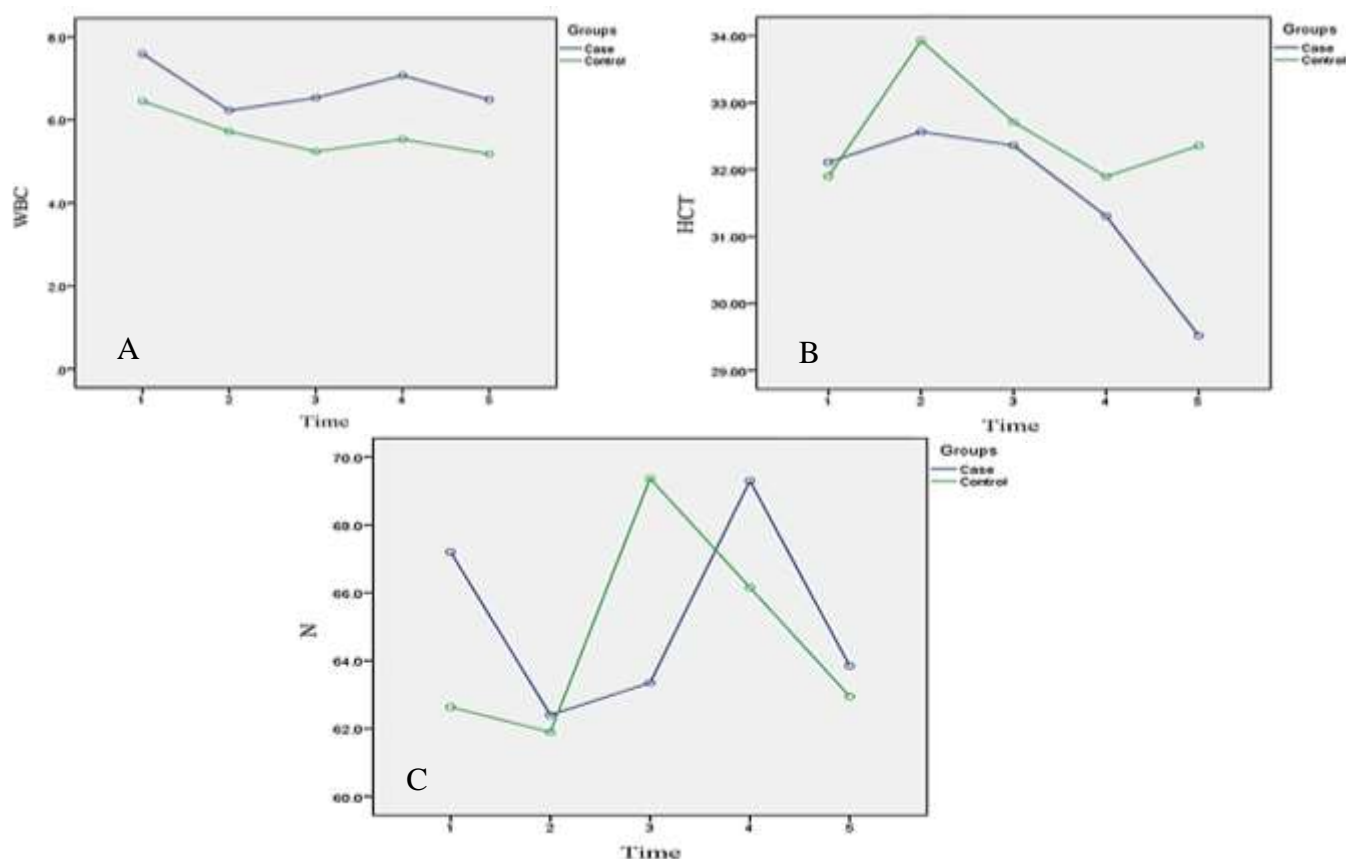


Figure 2. Changes Related to (A): WBC, (B): HCT, and (C): Neutrophil blood Indices for 5-Time Sampling in Test Group and Control Group.

Discussion

In the past few years, the incidence rate of invasive fungal infections has increased dramatically (15). Candidiasis infection is the most common oral fungal infection with a 75% incidence rate. This infection appears in various forms such as thrush, stomatitis, and angular cheilitis (15, 16). Immune system suppression due to chemotherapy is a condition that can lead to fungal infections (17). A previous study reported that *C. Albicans* is found in leukemia patients, while another study found this fungus in patients with solid tumors (18). The increase in chemotherapy treatments makes patients susceptible to candidiasis infections (19). Although consuming antifungal drugs eliminates these infections, taking them in the long-term causes the fungi to be resistant to treatment. In addition, taking these drugs leads to patients' weakness. Using natural products with health benefits such as probiotics is suggested to eliminate the above-mentioned risks (20). Kefir, as an alcoholic-lactic drink, is a probiotic product. Kefir grains, which are the yeast/bacteria fermentation starter, contain casein and species of *Lactobacillus*, *Saccharomyces*, *Streptococcus*, and other microbial materials (13). A number of studies evaluated the

nutritional and therapeutic properties of Kefir. Using this product for treating tuberculosis, cancer, and digestive disorders was prevalent even before the age of modern medicine (21). Meanwhile, it has been confirmed that Kefir has antibacterial and antifungal properties (13).

Based on the results, the topical administration of probiotic products such as Kefir had a positive effect on the chemotherapy patients and significantly decreased the prevalence of oral candidiasis in the test group, whereas a significant increase in *C. Albicans* was observed in the control group. Li et al. in their study on probiotic effects observed that the incidence rate of candidiasis was 100% before treatment. However, this rate was 34.6% in control (common antifungal treatment) and 8.21% in the test group (common antifungal treatment in addition to Kefir). This difference between the two groups was statistically significant, which is in line with our results. (22). Previous research on the antifungal effect of probiotics against pathogens causing candidiasis reported that these products showed antifungal activity against *Bacillus* spp, *Enterobacteriaceae*, and *Candida* spp. (23). In a study on candidiasis in mice, Ishijima et al. observed that treatment with probiotic products significantly affected oral

candidiasis. They suggested that this product can protect the oral environment, especially against candidiasis (24). Boarini et al. evaluated the bacterial probiotic effect on *Candida* and anti-*Candida* IgA. They reported a significant decrease in colonies of *Candida* formed in the oral cavity of the elderly. In addition, the level of anti-*Candida* antibodies increased significantly (1). The results of Teanpaisan et al. in Thailand are in agreement with the present study (2).

In a similar study conducted in China, in contrast to the control group, the test group showed a significant decrease regarding the count of *Candida* after 4 weeks. However, the two groups were not significantly different after 2 weeks (22). Blood indices such as WBC, RBC, neutrophil, platelets, and hematocrit counts were assessed in this study. Based on the results, there was no significant difference between the test and control groups regarding these indices. However, other studies showed a significant increase in WBC count in both groups. Finally, due to the relation between blood indices and the count of *C. Albicans* in both groups, it can be stated that using Kefir alone is not sufficient as an antifungal treatment. However, it can help in preventing fungal infections such as *Candida* in chemotherapy patients with immune system suppression. Based on the results, using probiotic products every day over a short period of time can reduce the load of oral *C. Albicans*. Meanwhile, using these products may reduce the effects of this fungus on other body organs and additionally reduce the risk of tooth decay.

Acknowledgments:

We are deeply grateful from Babol University of Medical sciences for financial supporting and chemotherapy Department of shahid Rajae babolsar hospital, Dr Ali Asghar Sefidgar lab, Dr Mostafa Vahedian and Dr Azin khodabakhshi.

Funding: This work was financially supported with grant number 9339011 by Babol University of Medical Sciences, Babol, Iran.

Ethics approval: P/Z/30 – 5032, Deat: January10, 2015. Ethic comitee of Babol University of medical scinces.

Conflict of Interests: The authors declare that they have no competing interests.

Authors' contribution: Moslemei D: study Design, selection, examination, management and fallow – up the patients. Latifi S: fallow – up the patients, Data collection, manuscript Draft of final report. Mehdizadeh M: Intellectual property and provide Idea, plan management, search artcils and texts, manuscript of final report.

References

- Mendonça FH, Santos SS, Faria ID, et al. Effects of probiotic bacteria on *Candida* presence and IgA anti-*Candida* in the oral cavity of elderly. *Braz Dent J* 2012; 23: 534-8.
- Teanpaisan R, Piwat S. *Lactobacillus paracasei* SD1, a novel probiotic, reduces mutans streptococci in human volunteers: a randomized placebo-controlled trial. *Clin Oral Investig* 2014; 18: 857-62.
- Tsang C, Chu F, Leung W, et al. Phospholipase, proteinase and haemolytic activities of *Candida albicans* isolated from oral cavities of patients with type 2 diabetes mellitus. *J Med Microbiol* 2007; 56: 1393-8.
- Hatcher H, Planalp R, Cho J, Torti F, Torti S. Curcumin: from ancient medicine to current clinical trials. *Cell Mol Life Sci* 2008; 65: 1631-52.
- Glick M, Greenberg MS, Lockhart PB, Challacombe SJ. *Burket's Oral Medicine*. 13th ed. Wiley 2021; pp: 94-101.
- Hupp JR, Ellis E, Tucker MR. *Contemporary oral and maxillofacial surgery*. 7th ed. St Louis: Mosby 2019; pp: 358. Available from: <https://s1.dentic.ir/book-3/145-contemporary-oral-maxillofacial-surgery.pdf>.
- Blasberg B, Greenberg MS. *Temporomandibular disorders*. 11th ed. Hamilton: BC Decker Inc 2008; pp: 223-55.
- Pinto TM, Neves AC, Leão MV, Jorge AO. Vinegar as an antimicrobial agent for control of *Candida* spp. in complete denture wearers. *J Appl Oral Sci* 2008; 16: 385-90.
- Teoh F, Pavelka N. How chemotherapy increases the risk of systemic candidiasis in cancer patients: current paradigm and future directions. *Pathogens* 2016; 5: 6.
- Pires FR, Santos EB, Bonan PR, De Almeida OP, Lopes MA. Denture stomatitis and salivary *Candida* in Brazilian edentulous patients. *J Oral Rehabil* 2002; 29: 1115-9.
- Matsuzaki T, Takagi A, Ikemura H, Matsuguchi T, Yokokura T. Intestinal microflora: probiotics and autoimmunity. *J Nutr* 2007; 137: 798S-802.
- Cildir SK, Germec D, Sandalli N, et al. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yogurt containing probiotic bacteria. *Eur J Orthod* 2009; 31: 407-11.
- Otles S, Cagindi Oe. Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. *Pakistan J Nutr* 2003; 2: 54-9.
- Kasra Kermanshahi R, Moatar F, Shadzi S, Mahdavi M. The antimicrobial and antifungal effects of Keffir in vitro. *J Babol Uni Med Sci* 2001; 3: 19-24. [in Persian]

15. Farah CS, Lynch N, McCullough MJ. Oral fungal infections: an update for the general practitioner. *Aust Dent J* 2010; 55 Suppl 1: 48-54.
16. Mahdavi Omran S, Rezaei Dastjerdi M, Zuashkiani M, Moqarabzadeh V, Taghizadeh-Armaki M. In Vitro Antifungal susceptibility of *Candida* species isolated from Iranian patients with denture stomatitis. *Biomed Res Int* 2018; 2018: 3086586.
17. Ghasemi Z, Hashemi SJ, Rezaei S, , et al. Molecular analysis of *Candida* species with emphasis on predisposing factors in cutaneous Candidiasis. *Jundishapur J Microbiol* 2017; 10: e41030.
18. Bodey GP, Mardani M, Hanna HA, et al. The epidemiology of *Candida glabrata* and *Candida albicans* fungemia in immunocompromised patients with cancer. *Am J Med* 2002; 112: 380-5.
19. Wingard JR. Infections due to resistant *Candida* species in patients with cancer who are receiving chemotherapy. *Clin Infect Dis* 1994; 19: S49-53.
20. Hatakka K, Ahola AJ, Yli-Knuuttila H, et al. Probiotics reduce the prevalence of oral *Candida* in the elderly—a randomized controlled trial. *J Dent Res* 2007; 86: 125-30.
21. Zourari A, Anifantakis E. Le kéfir. Caractères physico-chimiques, microbiologiques et nutritionnels. Technologie de production. Une revue. *Le Lait* 1988; 68: 373-92. [in French]
22. Li D, Li Q, Liu C, et al. Efficacy and safety of probiotics in the treatment of *Candida*-associated stomatitis. *Mycoses* 2014; 57: 141-6.
23. Polishchuk O, Koltukova N, Cherniavs' ka L, Reva O, Sorokulova I. The identification of freshly isolated strains, the causative agents of human candidiasis, and the search for effective antifungal probiotics. *Mikrobiol Z* 1999; 61: 45-53. [in Ukrainian]
24. Ishijima SA, Hayama K, Burton JP, et al. Effect of *Streptococcus salivarius* K12 on the in vitro growth of *Candida albicans* and its protective effect in an oral candidiasis model. *Appl Environ Microbiol* 2012; 78: 2190-9.