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Investigating the Influence of Probiotic Bacteria on Anemia and White Blood Cells Count in Cancer Patients Undergoing Chemotherapy and Radiation Therapy

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Introduction: Introduction: Probiotics are live microorganisms that are applied as drugs or food supplements and help to maintain healthy beneficial microbial balance in the digestive tract of a human or another host. Probiotics may help strengthen homeostasis and thus reduce side effects; associated with cancer treatment. Experimental evidence suggests that probiotics might have a beneficial effect on the toxicity of anticancer therapy.
Methods: In this study, the double-blind clinical trial was used to collect information and data. Eligible participants were randomly divided into two experimental groups (probiotic) and a control group (placebo). At the start of the study, during a meeting for all participants in the study, the benefits and importance of integrity and their collaboration were described. Subjects received one gram of probiotic or placebo for 60 days, along with information on the time and how to use it. Also, all participants avoided the use of yogurt and any supplementary probiotic products during the study period to avoid any inaccuracy in evaluating the involved factors. At the end of 60 days, the blood samples of the subjects were examined. The number of white blood cells (WBCs), blood hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were measured and analyzed. People who did not follow the instructions for any reason were excluded from the study.
Results: The effect of probiotics on reducing the complications of chemotherapy in patients with gastrointestinal cancer was studied. The statistical results did not show much difference in the increase of these factors before and after the administration of the drug but clinical examination of these factors showed that probiotics can increase blood factors. Probiotics may have a positive effect on the increase in blood factors level (MCH, MCV, HCT, HGB, WBC) by increasing the duration of receiving probiotics.
Conclusions: In this research, the effects of probiotics on the increase of blood factors were investigated for the first time. Although statistical analysis of the results showed that probiotics did not affect the increase of these factors, clinical examination of the results showed that probiotics can increase blood factors. Therefore, by changing the appropriate dose, time, and diet, the duration of probiotics may positively affect the level of blood factors (MCH, MCV, HCT, HGB, and WBC) and improve the living conditions in these patients.

INTRODUCTION

It is supposed, that complementary and alternative medicine, like vitamins, minerals, herbs, and other dietary supplements including probiotics, is used by more than 80% of cancer patients [1]. The concept of probiotics was raised in the early 20th century by Elie Metchnikoff, a Russian Nobel laureate who discovered that some strains of bacteria in human bodies were beneficial to various human processes. These excellent bacteria were named probiotics [2]. Probiotics are live microorganisms that as drugs or food supplements help to maintain healthy beneficial microbial balance in the digestive tract of a human or other hosts. There is rising interest in probiotics which is also reflected in several published works in this area. They are tested in multiple indications including gastrointestinal disorders like prevention and treatment of infectious and antibiotic-induced diarrhea, treatment of liver insufficiency, lactose intolerance, inflammatory bowel disease, and irritable bowel syndrome [3]. Probiotics seem to be important due to their association with intestinal microflora and stimulation of the immune system. They are non-pathogenic bacteria that rarely lead to illness in healthy people [4, 5]. Probiotics are beneficial microorganisms that cause beneficial effects on the host's health when used in humans or animals by affecting the microbial flora of the body. Most of the probiotics belong to a large group of bacteria in the human intestinal microbial flora, and they have a harmless commensalism life. The most common probiotic microorganisms are divided into two groups of bacteria and fungi [4]. Among probiotic bacteria, Lactobacillus and Bifidobacterium have more efficacy and among yeast, Saccharomyces boulardii has the highest effects. Although species of Enterococcus, Streptococcus, and E.coli are also used for this purpose. Most of the Lactobacillus and Bifidobacterium bacteria are recognized as safe. Except for Streptococcus and Enterococcus, other acid-causative bacteria are rarely found to be pathogenic to humans and animals, and their use has long been proven to cause no adverse effects [6, 7]. Probiotics, in particular bacteria, have beneficial effects in their hosts, such as balancing intestinal flora, preventing the binding of pathogenic bacteria to the intestinal mucosal wall, increasing the specific and non-specific immune response, suppressing inflammation, reducing the occurrence of cancer (by producing enzymes, inducing apoptosis, and controlling the toxicity of carcinogens), and preventing/treating diarrhea. Certain probiotics also have anticarcinogenic activity by regulating the homeostasis of the intestine epithelial cells and immune response [8, 9].

Studies on germ-free animals have shown that these microorganisms play an important role in the development and maturation of the central nervous system (CNS) and enteric nervous system (ENS) and also affect brain function [10]. One of the obvious side effects of chemotherapy and radiation therapy is digestive disorders due to the destruction and collapse of natural flora [11, 12], abdominal pain, fatigue, malnutrition, loss of water and electrolyte, and disturbed complete blood count (CBC) (especially hemoglobin and WBC level) [13, 14]. Bacterial therapy is an alternative therapeutic approach that leads to competition for the replacement of microorganisms [15, 16]. One of the most common bacterial therapies in the clinic is the use of probiotics. According to the definition of WHO, a probiotic is a food supplement; containing living bacteria with many benefits to the digestive balance [6, 17]. Probiotics may help strengthen homeostasis and thus reduce the side effects associated with cancer treatment. Experimental and some clinical evidence suggest that lactic acid bacteria might have a beneficial effect on the toxicity of anticancer therapy. Reducing the side effects of chemotherapy and radiation therapy is an issue that is critical in improving the quality of life and hope for treatment in patients with cancer [18, 19]. Therefore, due to the apparent utility of these microorganisms, clinical studies seem necessary in this regard. This study aimed to investigate the effect of probiotics on the reduction of complications caused by chemotherapy.

METHODS

This study is a double-blind randomized clinical trial that was approved by the ethics committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1395.265) on 25-7-2016. Participants were randomly divided into two groups: (experimental/probiotic) and a control group (placebo) after obtaining the informed consent. At the beginning of the study, the benefits and importance of honesty and their co-operation were described. Subjects received 1 gr probiotic (5-6×10^9 CFU/mL of probiotic from Tak-gene Company) or placebo per day for 60 consecutive days. The sampling time was 12 months with a prosperous community of patients with gastrointestinal cancer. A total of 40 participants from both sexes were selected and randomly assigned to experimental (probiotic) and control (placebo) groups. Subjects received one gram of probiotic (company) or a placebo for 60 days, along with information on how to use it. All participants avoided the use of yogurt and any supplemental probiotic product during the study period to avoid any inaccuracy in the evaluation of the studied factors. Blood tests were performed on subjects before and after 60 days. Serum levels of white blood cells (WBCs), blood hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were measured. Also, by distributing the questionnaire among the subjects, the reduction of digestive disorders was analyzed. People who have used a specific drug during the study or those who have not followed the instructions for any reason were excluded.

RESULTS

The outcomes of each group were analyzed before and after using the drug and placebo (Table 1).

As shown in Table 1, in the experimental group, the mean±standard deviation (SD) of the MCH, HCT, HGB, and WBC indices before and after probiotic application were 27.79±3.54 vs. 28.55±2.39, 36.82±5.85 vs. 38.28±5.67, 11.71±1.56 vs. 12.59±1.78, and 5.66±2.72 vs. 7.61±2.83, respectively that indicates an increase in these indices after treatment. The mean±SD of MCV before and after probiotic application were 85.63±9.24 and 83.76±12.98, respectively which indicates a decrease in this index after receiving the drug. In the placebo group, the mean±SD of the MCH, MCV, and HCT indices before and after the intervention indicated an increase in these indices after receiving the placebo (28.98±4.99 vs. 30.04±3.77, 87.65±11.82 vs. 9.18 ±8.88, and 37.34±4.84 vs. 35.68±3.48). The respective mean±SD of HGB and WBC levels were (12.28±1.73 vs. 11.75±1.48 and 5.49±1.60 vs. 5.74±2.56); indicating a decrease in this index after receiving the placebo.

Kolmogorov-Smirnov test was performed to confirm the hypothesis that all variables have a normal distribution. As seen in Table 2, H0 is confirmed in all variables. This means that all variables have a normal distribution.

Table 1: Mean and Standard Deviation of Index Scores in Experimental and Control Groups[a]

	Patients, No.	Before		After	
		Mean	Standard Deviation	Mean	Standard Deviation
MCH					
Placebo	7	28.98	4.99	30.04	3.77
Drug	9	27.79	3.54	28.55	2.39
MCV					
Placebo	7	87.65	11.82	91.18	8.88
Drug	9	85.63	9.24	83.76	12.98
HCT					
Placebo	7	37.34	4.84	35.68	3.48
Drug	9	36.82	5.85	38.28	5.67
HGB					
Placebo	7	12.28	1.73	11.75	1.48
Drug	9	11.71	1.56	12.59	1.78
WBC					
Placebo	7	5.9	1.60	5.74	2.56
Drug	9	5.66	2.72	7.61	2.83

[a] Abbreviations: HCT, hematocrit; HGB, blood hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; WBCs, white blood cells

Table 2: Results of the Kolmogorov-Smirnov Test[a]

	Confidence Level		Assumption
	Drug	Placebo	
MCH	0.939	0.523	H0
MCV	0.966	0.488	H0
HCT	0.981	0.974	H0
HGB	0.880	0.896	H0
WBC	0.736	0.051	H0

[a] Abbreviations: HCT, hematocrit; HGB, blood hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; WBCs, white blood cells

Inferential statistics were performed to determine the homogeneity of the two groups of placebo and drug before the start of treatment. The indices of both were evaluated based on the standard one-sample t-test test which is summarized in Table 3.

Table 3: The Results of One-Sample T-Test to Compare the Indices in the Placebo and Drug Groups With the Baseline[a]

	T Value	Degree of Freedom	Confidence Level (P Value)	Mean Difference	Result
MCH					
Placebo	1.517	8	0.168	1.790	Accepted
Drug	1.580	6	0.165	2.984	Accepted
MCV					
Placebo	1.829	8	0.105	5.630	Accepted
Drug	1.714	6	0.137	7.655	Accepted
HCT					
Placebo	0.422	8	0.684	0.820	Accepted
Drug	0.734	6	0.491	1.342	Accepted
HGB					
Placebo	0.557	8	0.593	0.288	Accepted
Drug	0.436	6	0.678	0.286	Accepted
WBC					
Placebo	1.835	8	0.104	1.663	Accepted
Drug	2.473	6	0.048	1.497	Rejected

[a] Abbreviations: HCT, hematocrit; HGB, blood hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; WBCs, white blood cells

Based on the results of the one-sample t-test, none of the groups showed any significant difference in specific indices (MCH, MCV, HCT, HGB) with the minimum standard or baseline (assertion of zero assumption) but a significant difference was observed in the WBC index between the mean of the drug group and the baseline which makes this index eliminated. To analyze this hypothesis, multi-path covariance analysis (MANCOVA) was used.

Table 4 shows the level of significance ($P<0.05$) which indicates that the homogeneity condition of the variance-quantification matrix is not observed ($F=0.984$; $P=0.001$).

Table 4: The Evaluation of Variance-Covariance Homogeneity of All Indices

BOX S M	F	Degree of Freedom 1	Degree of Freedom 2	Confidence Level
0.45	3.984	10	786.795	0.001

Therefore, covariance analysis could not be used. On the other hand, because the distribution of data was normal, and both groups were homogeneous, in order to compare the two groups, first, the difference in the scores of the indicators before and after receiving the drug and placebo is calculated, and finally, the results of the two groups are analyzed using the independent t-test. Independent t-test was performed to compare indicators in two groups before receiving drug and placebo (Table 5).

Table 5: Independent T-Test Results to Compare Indices in Two Groups Before Treatment[a]

	Patients, No.	T Value	Degree of Freedom	Confidence Level (P Value)	Mean Difference	Result
MCH		0.249	712/13	0.807	0.299	Accepted
Placebo	7					
Drug	9					
MCV		1.198	14	0.251	5.399	Accepted
Placebo	7					
Drug	9					
HCT		1.167	14	0.263	3.112	Accepted
Placebo	7					
Drug	9					
HGB		1.687	14	0.114	1.406	Accepted
Placebo	7					
Drug	9					
WBC		0.719	14	0.484	1.696	Accepted
Placebo	7					
Drug	9					

[a] Abbreviations: HCT, hematocrit; HGB, blood hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; WBCs, white blood cells

Independent t-test results, before treatment, showed that there was no significant difference between the two groups in none of the indices. Independent t-test was performed to compare indicators in two groups after receiving drug and placebo.

Table 6: Independent T-Test Results to Compare Indices in Two Groups After Treatment[a]

	Patients, No.	T Value	Degree of Freedom	Confidence Level (P Value)	Mean Difference	Result
MCH		0.969	14	0.349	1.495	Accepted
Placebo	7					
Drug	9					
MCV		1.291	14	0.218	7.422	Accepted
Placebo	7					
Drug	9					
HCT		1.059	14	0.308	2.592	Accepted
Placebo	7					
Drug	9					
HGB		0.995	14	0.337	0.832	Accepted
Placebo	7					
Drug	9					
WBC		0.601	14	0.558	1.862	Accepted
Placebo	7					
Drug	9					

[a] Abbreviations: HCT, hematocrit; HGB, blood hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; WBCs, white blood cells

Independent t-test results showed that there was no significant difference between the drug and placebo groups in all the indices. Therefore, the zero assumption is confirmed, which is, the desired drug can not affect the decrease or increase of the indices studied.

DISCUSSION

In this research, the effects of probiotics on the increase of blood factors were investigated for the first time. The mean and standard deviation of WBC, HGB, HCT, MCV and MCH increased after receiving the drug. The results of Kolmogorov-Smirnov test showed that all variables (MCH, MCV, HCT, HGB, and WBC) have normal distribution. Independent t-test showed that there was no significant difference between the placebo and the drug group in all indices. The results of the box test which was performed to investigate the assumption of homogeneity of the variance-covariance matrix in all indicators showed that the significance level was $P<0.05$ which indicates that the variance-covariance matrix was not homogeneous ($F=3.984$ and $P=0.001$). Therefore, to compare the two groups, the difference between the scores of the before and after indices were calculated, and then the results of the two groups were examined; using an independent t-test. Hence, the mean and SD of the index scores in the two groups of placebo and drug were changed. To compare the two groups and their effects, an independent t-test was used. Therefore, the desired drug cannot affect the reduction or increase of the indices studied. So far, little research has been done on the effect of probiotics on the reduction of anemia induced by chemotherapy.

Rodgers et al., showed that probiotics were effective in treating chemotherapy-induced anemia [20]. Mego et al., showed that probiotics were effective in treating chemotherapy-induced neutropenia [21]. In this study, although statistical analysis of the results showed that probiotics did not affect the increase of these factors, clinical examination of the results showed that probiotics can increase blood factors. Probiotics may have a positive effect on the increase in blood factors levels (MCH, MCV, HCT, HGB, WBC) by increasing the duration of receiving probiotics.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS APPROVAL

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RCT REGISTRATION CODE

Awaiting judgment With Trial ID 64395

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