

Effect of supplements: Probiotics and probiotic plus honey on blood cell counts and serum IgA in patients receiving pelvic radiotherapy

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Background: Radiotherapy is frequently used in treatment approaches of pelvic malignancies. Nevertheless, it has some known systemic effects on blood cells and the immune system that possibly results in their susceptibility to infection. Probiotics are live microbial food ingredients that provide a health advantage to the consumer. Honey has prebiotic properties. The aim of this clinical trial was to investigate probable effects of probiotic or probiotics plus honey on blood cell counts and serum IgA levels in patients receiving pelvic radiotherapy. **Materials and Methods:** Sixty-seven adult patients with pelvic cancer were enrolled. Patients were randomized to receive either: (1) Probiotic capsules (including: *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum*, and *Streptococcus thermophiles*) ($n = 22$), (2) probiotic capsules plus honey ($n = 21$) or (3) placebo capsules ($n = 24$) all for 6 weeks. Blood and serum samples were collected for one week before radiotherapy and 24-72 h after the end of radiotherapy. **Results:** White blood cells (WBC), red blood cells (RBC), platelet counts, and serum IgA level were not significantly changed in patients taking probiotic (alone or plus honey) during pelvic radiotherapy. The mean decrease in RBC count was 0.52, 0.18, and 0.23×10^6 cells/ μ L, WBC count was 2.3, 1.21, and 1.34×10^3 cells/ μ L and platelet count was, 57.6, 53.3, and 66.35×10^3 cells/ μ L for the probiotic, probiotic plus honey, and placebo groups, respectively. The mean decrease of serum IgA was 22.53, 29.94, and 40.73 mg/dL for the probiotic, probiotic plus honey, and placebo groups, respectively. **Conclusion:** The observed nonsignificant effect of probiotics may be in favor of local effects of this product in the gut rather than systemic effects, however, as a trend toward a benefit was indicated, further studies are necessary in order to extract effects of probiotics or probiotic plus honey on hematologic and immunologic parameters in patients receiving pelvic radiotherapy.

Key words: Blood cell counts, probiotic, prebiotic, honey, radiotherapy, pelvic cancer, serum IgA

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INTRODUCTION

Radiotherapy is a treatment for many types of pelvic malignancies, which uses high-energy beams of radiation to kill the cancer cells. It may be done alone or in combination with other treatments such as surgery or chemotherapy. The lethal effect of radiation is not restricted to the cancer cells. A minority of normal human cells such as the undifferentiated progenitors of the hematopoietic system and mucosa cells are sensitive to radiation. Mature lymphocytes are among the most radiosensitive human cells.^[1]

Radiotherapy has systemic effects on immunity using different mechanisms. Experiences from humoral immunity effects of radiation on patients with Hodgkin's lymphoma have shown a reduction of immunoglobulin values of IgM, IgA, and IgG with radiotherapy. A study showed values remained mostly within the lower normal range and a mean period of 2, 8, and 12 months were needed until IgM, IgA, and IgG recover, respectively.^[2] Moreover, radiation of the gastrointestinal tract may compromise the physical barrier of the mucosa. In certain circumstances, disruption of antimicrobial secretions of the gut resulted in an invasion by local flora and lead to bacteremia and candidemia.^[3,4] Studies

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suggest a healthy diet such as ingestion of probiotics can be advantageous. These are live microorganisms that include bacteria (e.g., *Lactobacillus* and *Bifidobacterium* species) or yeasts (e.g., *Saccharomyces boulardii*). Probiotics usage can restore the healthy flora of the gut, improve intestinal function,^[5] and reduce the incidence of diarrhea during pelvic radiotherapy.^[6-9] The immune properties of probiotics have been related to signaling the immune system through innate cell surface pattern recognition receptors or directly by lymphoid cell activation. In some cases, this action has shown to modulate local and systemic immune responses.^[10,11] Positive effects of probiotics on IgA secretion of respiratory^[12] and gastrointestinal^[13,14] tract have been reported. Noticeably, no significant side effects have been reported for intake of recommended doses of probiotics except for mild abdominal pain or bloating that are usually self-limited.^[15]

“Prebiotics” are food ingredients that stimulate the growth and/or activity of probiotics in ways claimed to be beneficial to health.^[16] Honey has been valued in Scriptures and mentioned in ancient medical literature such as in The Canon of Medicine by Avicenna (980-1037 AC). It has acknowledged anti-inflammatory, antioxidant, antitumor,^[17-19] and effective “prebiotic” properties.^[20-22] Therefore, it may be used as a new remedy that is almost free from side effects.

As described above the benefits of probiotics on gut mucosa is well-known. However, whether patients receiving pelvic radiotherapy benefit from the probiotics and prebiotics by systemic humoral alterations have not been investigated in pelvic radiotherapy. The present study was conducted to assess the possible positive effects of probiotics alone or using concurrently with honey on blood cell counts and serum IgA level in patients undergoing pelvic radiotherapy.

MATERIALS AND METHODS

This randomized clinical trial was performed on 67 patients diagnosed with pelvic cancers (colorectal, prostate, endometrial, bladder, ovary, cervical, and bone) that were referred to Radiation Oncology Clinic at Sayyed-Al-Shohada Hospital, Isfahan, Iran, from October 2012 to May 2013. The treatment protocol was explained to patients, and they were given informed consent to participate. The exclusion criteria were dissatisfaction with the continuation of treatment, immunosuppression treatment and not referring for receiving treatment regime.

The sample size was calculated 21 persons in each group using following equation with 80% power, 95% confidence interval, and $d = 0.6$ ($P_1 = 0.32$, $P_2 = 0.09$ according results of Chitapanarux et al. study).^[6]

$$n = (Z_{1-\alpha/2} + Z_{1-\beta})^2 (P_1 (1-P_1) + P_2 (1-P_2)) / d^2$$

Randomization was used to allocate patients to three groups: (1) Taking probiotics ($n = 22$); (2) taking a probiotic combination with honey ($n = 21$); and (3) taking placebo ($n = 24$), [Figure 1].

All patients were irradiated with conventional two-dimensional box fields (62.5%) and parallel opposed fields (37.5%) to a total dose ranged from 4000 to 6200 centiGrays (mean = 4799.6, standard deviation [SD] = 522.5) (2 Gy/day) with 18 MV (84.5%) and 9 MV photon beams (10.3%) and Co60 (5.2%). The upper edge of the AP field varied from the third lumbar vertebra to the first sacral bone and extended to the lower edge of the pubic bones and laterally, covering a 1.5-2 cm of pelvic inlet rim but this also varied according to the abdominopelvic disease. Thirty-five percent of patients were irradiated with local parallel opposed boost fields (35.7%). Boost dose ranged from 260 to 2000 centiGrays (mean = 1210.0, SD = 662.3). Anteroposterior field dimensions ranged from 64 cm to 270 cm (mean = 201.9, SD = 45.3), and the dimensions of the lateral ranged from 84 cm to 256 cm (mean = 139.1, SD = 49.4) and boost fields were from 64 cm to 144 cm (mean = 105.9, SD = 21.3). Patients received pelvic radiotherapy for 5 weeks.

The probiotic capsules “LactoCare” (Zist Takhmir Company, Tehran, Iran) including: *Lactobacillus casei* 1.5×10^9 CFU, *Lactobacillus acidophilus* 1.5×10^{10} CFU, *Lactobacillus rhamnosus* 3.5×10^9 CFU, *Lactobacillus bulgaricus* 2.5×10^8 CFU, *Bifidobacterium breve* 1×10^{10} CFU, *Bifidobacterium longum* 5×10^8 CFU, and *Streptococcus thermophilus* 1.5×10^8 CFU per 500 mg have been used. The natural coriander honey used in this study was obtained from the region of the Zagros Mountains (Khansar) of Iran (Keshtzare Sabz Company, Khansar, Iran) and its quality (in terms of reducing sugars, total sugars,

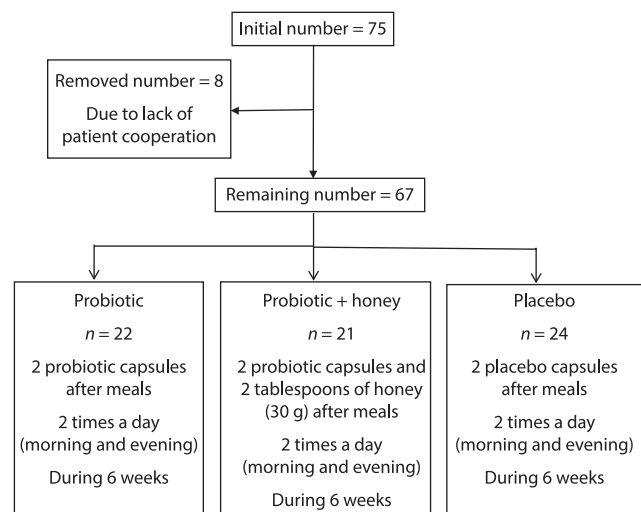


Figure 1: Illustrates the prescription regimens

sucrose, glucose, fructose, fructose than glucose, and amylase) was confirmed by the Food and Drug Administration Isfahan University of Medical Sciences, and was in accordance with the National Standard No.92 (Iranian National Standards Organization). Placebo capsules are manufactured by the Zist Takhmir Company, Tehran, Iran (Capsules contain 500 mg of corn starch). The placebo capsules were the same size, shape, and color as the probiotic capsules.

A complete blood count test including: Red blood cell (RBC), white blood cell (WBC) and the platelet count was performed one week prior to beginning of radiotherapy and 24-72 h after last week of radiotherapy by cell counter machine model H1 on blood sample of patients. The IgA level of the patient's sera sample was measured by autoanalyzer machine Hitachi model 902 and quantitative determination of human immunoglobulin A kit (Bionik company, Tehran, Iran).

Data were analyzed using: Chi-square, Fisher's exact, and one-way ANOVA test. Postanalysis was done by least significant difference method in order to do a pairwise comparison (treatment group vs. placebo group). The $P < 0.05$ were considered as statistically significance. Statistical analyses were performed using SPSS v.16 (SPSS Inc., Chicago, IL, USA).

Mean and standard deviations of the quantitative values and the mean and percentage of qualitative variables were reported.

The study was approved ethically by the Ethical Committee of the Isfahan University of Medical Science and was registered in the Iranian Registry of Clinical Trials (IRCT2015030421338N1).

RESULTS

The patient was aged between 20 and 85 years, mean (SD) age = 62 (14.8) years, 39 patients (58.2%) were male, and 28 of them (41.8%) were female. There were no significant differences in age and sex between patients in the three groups. Patient characteristics are presented in Table 1.

The blood analysis performed before and after the radiotherapy showed that the parameters of clinical relevance including red and WBCs and platelet count were within their respective ranges of normality. As seen in Table 2, the mean of cell blood counts at after radiotherapy reduced in all groups. These changes were not statistically significant. The patients who take probiotic plus honey have the least reduction in red and WBCs and platelet count compared to other groups.

The mean of serum IgA level decreased after radiotherapy: From 216.1 (SD: 87.7) to 193.5 (89.6) mg/dL in the probiotics

group, from 236.3 (58.6) to 206.3 (64.3) mg/dL in the probiotics plus honey group, and from 264.3 (96.3) to 205.6 (79.2) mg/dL in the placebo group.

These reductions were not statistically significant when compared with placebo group (P values were 0.15 and 0.29 for the probiotic and probiotic plus honey group, respectively). The patients who take probiotic have the least reduction of serum IgA level compared to other groups [Figure 2].

DISCUSSION

Since, it has been proposed that probiotics modulate the intestinal immune response through the stimulation and modulation of certain cytokines and IgA secretion,^[14] the aim of the present study was to evaluate and compare the systemic effects of probiotics on blood cells and serum IgA. This study did not show a statistical significance effect of

Table 1: Patient characteristics

Character	Study arms			P
	Probiotic (n = 22)	Probiotic - honey (n = 21)	Placebo (n = 24)	
Age (mean \pm SD)	63.73 \pm 15.09	57.86 \pm 17.48	64.17 \pm 11.69	0.44 ^a
Gender				
Male	14 (20.9)	8 (11.9)	17 (25.4)	0.15 ^b
Female	8 (11.9)	13 (19.4)	7 (10.4)	
Cancer type				
Colorectal	6 (9.0)	9 (13.4)	9 (13.4)	0.42 ^c
Prostate	6 (9.0)	3 (4.5)	6 (9.0)	
Endometrial	3 (4.5)	5 (7.5)	2 (3.0)	
Bladder	4 (6.0)	0	4 (6.0)	
Ovary	2 (3.0)	1 (1.5)	1 (1.5)	
Cervical	1 (1.5)	1 (1.5)	2 (3.0)	
Bone sarcoma	0	2 (3.0)	0	
ChRt (yes)	8 (11.9)	9 (13.4)	9 (13.4)	0.9 ^b

Data in parentheses are a percentage of total patients. ^aOne-way ANOVA test;

^bChi-square test and ^cFisher's exact test. ChRt = Chemoradiation therapy; SD = Standard deviation

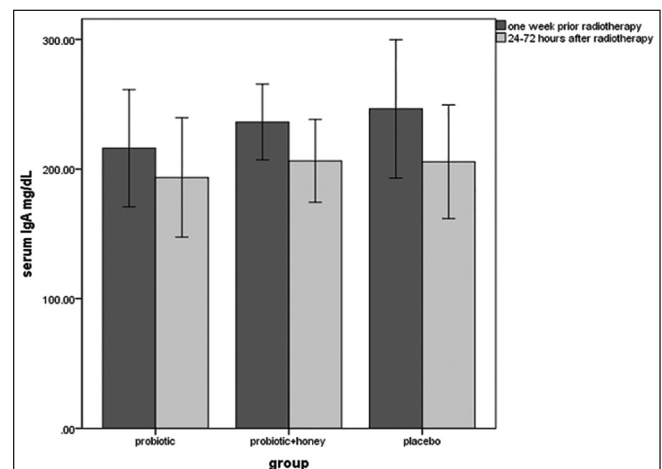


Figure 2: Comparison of serum IgA level between groups values show mean. Error bars and the 95% confidence intervals

Table 2: Patient's blood cell counts before and after radiotherapy

Blood cell	Placebo (n = 24)	Probiotic (n = 22)	P*	Probiotic + honey (n = 21)	P*
RBC (10 ⁶ /μL)					
Before	4.56 (0.69)	5.05 (0.79)	0.22	4.69 (0.54)	0.82
After	4.33 (0.74)	4.53 (1.03)		4.51 (0.54)	
Difference	-0.23 (0.41)	-0.52 (1.08)		-0.18 (0.39)	
WBC (10 ³ /μL)					
Before	7.52 (2.43)	7.33 (2.25)	0.24	6.90 (3.14)	0.86
After	6.17 (2.18)	5.03 (2.09)		5.68 (2.94)	
Difference	-1.34 (1.84)	-2.3 (2.47)		-1.21 (2.57)	
PLT (10 ³ /μL)					
Before	318.6 (112.4)	320.8 (96.7)	0.79	303.2 (73.1)	0.68
After	252.2 (76.1)	263.8 (123.8)		249.8 (61.6)	
Difference	-66.3 (81.2)	-57.6 (136.3)		-53.3 (63.9)	

Data are presented by mean (SD). Before = 1-week prior to beginning radiotherapy; After = 24-72 h after last week of radiotherapy. The difference = mean value of "after" subtracted from "before". *P = Level of significance for postanalysis (the control group compared with the treatments groups). RBC=Red blood cell; WBC = White blood cell; PLT = Platelet; SD = Standard deviation

probiotic intake during treatment time on WBC counts and serum IgA levels. This was compatible with some other studies that failed to show a significant immunologic boost translated to a possible protective effect of these agents. A double-blind study was performed on 45 elderly patients fed by enteral tube who were divided into a probiotic group and placebo group and a tendency toward an increase of serum IgA was seen by probiotic intake ($P = 0.085$ at week 4 and $P = 0.070$ at week 16).^[23] Another clinical trial on children with allergic rhinitis also showed no significant statistical differences of immunological or blood cell variables from the baseline and 12 weeks later when using *Lactobacillus salivarius*.^[24] Similarly in a randomized, double-blind placebo-controlled study on pre-school children that the total serum IgA and total salivary IgA were measured. The total serum IgA was not significantly increase in the probiotic group, but the salivary IgA level significantly increased in this group.^[25] Conversely, a double-blind, randomized controlled trial that was conducted in an intensive care unit on 28 critically ill patients showed that the patients who received probiotics had a statistically significant larger increase in systemic IgA and IgG concentrations compared with the patients who received placebo ($P < 0.05$).^[26]

Since, the present study is among the first clinical studies that investigated the effect of probiotics and probiotic plus honey on cells blood and serum IgA in the patients undergoing pelvic radiotherapy, data for making a practical assumption is scarce.

The fact that commensal bacteria and probiotics interact with host immunity is well-established but the molecular pathways of this "bacteria-host crosstalk" are not precisely known.^[27] Thus, whether an interaction between acute radiation damage of mucosal lineage and the cross-talk exists or not cannot be concluded evidently.

Considering the high incidence of cancer in the pelvic area and radiation therapy done for a significant number of these patients, reducing complications and improvement of the quality of life is of importance. Patients treated with usual volumes of conventional two-dimensional pelvic fields are not supposed to induce opportunistic susceptibility pancytopenia and routine check of complete blood cell is not always necessary.^[28] In addition, based on the research findings, the usage of probiotics in patients receiving pelvic radiotherapy is safe and has well-established benefits such as control and prevention of radiation-induced diarrhea.^[6-9]

Main limitations of this study were the small sample size that made subgroup analysis inconclusive especially in patients receiving chemotherapy and patients with different types of cancers. Also, the limitations about study duration and difficulties about patients' cooperation could be regarded.

Finally, the nonsignificant results of the study may contribute to localized effects of probiotics and the lack of clinically evident systemic effects of these products on blood cells and serum IgA. Other immunological factors, as well as WBCs differentiation on a quantitative time plot, is suggested to be analyzed in future well-designed studies that may enlighten the radiobiological and immunological aspects of this entity and drive to practical implications.

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Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTION

HMT; contributed in the conception and design of the work, conducting the study, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MRKh; contributed in the design of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. SMH; contributed in the analysis and interpretation of data, revising the draft, approval of the final version of the manuscript. FM; contributed in the conception and design of the work, approval of the final version of the manuscript, and agreed for all aspects of the work. HM; contributed in the drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MR; contributed in the conception and design of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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