

# Evaluation of the effect of probiotics on celiac patients

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## ABSTRACT

**Aim:** It was aimed to evaluate the effect of yogurt consumption containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* probiotic bacteria on the celiac markers, biochemical values and clinical symptoms of celiac diseases.

**Material and Method:** Fifty celiac patients with positive anti-tissue transglutaminase (anti-tTG) IgA values were evaluated prospectively. The patients were divided into two groups. One group was recommended only gluten-free diet (GFD), while the other group was recommended to eat 200 mL probiotic yogurt containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* daily in addition to GFD. The change in biochemical values, vitamin levels, celiac markers and clinical symptoms of both groups was examined after three months of yogurt consumption.

**Results:** Anti-tTG IgA, anti-deamidated gliadin peptide antibody (anti-DGP) IgA and anti-endomysium antibody (EMA) levels of the two groups decreased significantly after three months in both groups. There was a significant decrease in EMA levels in the probiotic yogurt+GFD group ( $p=0.025$ ) when compared with GFD group. Vitamin D and ferritin levels of the probiotic yogurt+GFD group increased significantly after three months (respectively  $p=0.004$ ;  $p=0.039$ ). Folate, vitamin D and iron levels of GFD group increased significantly (respectively  $p=0.002$ ;  $p=0.007$ ;  $p=0.007$ ). In both groups, clinical symptoms significantly decreased after three months. There was a significant decrease in the abdominal pain symptoms in the probiotic yogurt+GFD group when compared with GFD group ( $p=0.012$ ).

**Conclusion:** Gluten-free diet and consumption of yogurt containing *Streptococcus thermophilus*, *L. delbrueckii* ssp. *bulgaricus* and *Lactobacillus acidophilus* were found to be statistically significant in decreasing EMA levels and abdominal pain from irritable bowel syndrome (IBS) type symptoms of celiac patients when compared with GFD only.

**Keywords:** Celiac disease, anti-endomysium antibody, gluten-free diet, *Lactobacillus*, probiotic

## INTRODUCTION

Celiac disease (CD) is an immune-mediated chronic inflammatory disease manifested by chronic gluten sensitivity in people (1). The definitive diagnosis of CD in adults is made by small intestine biopsy, together with serological and genetic markers. Serological tests used in the diagnosis of CD are anti-deamidated gliadin peptide antibody (anti-DGP), anti-endomysium antibody (EMA), and anti-tissue transglutaminase (anti-tTG) antibodies. Serological tests are used to monitor compliance with a gluten-free diet and screen people at risk for CD (2). Gluten-free diet (GFD) is the only effective treatment for CD (1).

Probiotic microorganisms show beneficial effects by regulating the intestinal microbial balance. It has been shown that probiotics contribute to CD recovery by repairing the intercellular barrier function, inducing the hydrolysis of gliadin, anti-inflammatory effect, and reducing irritable bowel syndrome (IBS) symptoms (3-7).

In this study, we aimed to evaluate the effect of probiotic riched home-made yogurt consumption containing *Streptococcus*

*thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* probiotic bacteria, for three months; on celiac markers, biochemical values, and clinical symptoms of CD in patients diagnosed with CD on GFD. Our study is the first pilot study showing the effects of probiotic yogurt consumption on the prognosis of CD.

## MATERIAL AND METHOD

In this study, celiac patients with positive anti-tTG IgA values who applied to the adult gastroenterology clinic between December 1, 2018-August 31, 2019, were evaluated. The study was planned as a prospective case-control study.

Fifty patients aged between 18-72 years were included. Patients were divided into two groups. Gluten-free diet was recommended for both groups. The study group was recommended to consume 200 ml of probiotic yogurt every day in addition to the GFD.

Age, gender, initial and 3<sup>rd</sup> month hemoglobin (Hb), ALT, AST, ALP, albumin, calcium, iron, ferritin, vitamin B12, folate, vitamin D, anti-tTG, anti-DGP and EMA values, and clinical symptoms (diarrhea, constipation, abdominal pain, bloating, reflux) were evaluated in both groups.

Probiotic yogurt containing a total of 4x10<sup>11</sup> *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* probiotic bacteria per 200 grams was used. BÜYÜYO Probiotic Yogurt (Danem Milk and Milk Products Co. Ltd., Technoport Building Süleyman Demirel University, Isparta, Türkiye) was provided to produce probiotic yogurt. Patients made their yogurt at home by producing this yogurt.

Total IgA levels were studied with the Roche/Hitachi Cobas system by an immunoturbidimetric method with the IGA-2 kit (Roche Laboratory Systems, Germany). Patients with IgA levels below 0.70 g/L were considered IgA deficient and were excluded from the study.

Anti-tTG IgA and anti-DGP IgA antibodies were studied using Delta Biologicals (Italy) commercial kits based on enzyme immunoassay, an automated study was performed on Mago Touch (Italy) micro ELISA device. Antibodies were evaluated as positive when anti-tTG IgA value was  $\geq 20$  IU/ml, and antibodies were evaluated as positive when anti-DGP IgA value was  $>30$  AU/ml. Anti-endomysium IgA antibody was studied by the indirect immune fluorescence method. It was evaluated as negative or 1, 2, 3, or 4 positive at 20X and 40X magnification by Biochipler brand immunofluorescence microscope (Eurostar III plus, Özmen Medical Laboratory Diagnostics Inc.).

The data were analyzed with the "SPSS for Windows 22.0 (Statistical Package for Social Sciences Inc, Chicago, IL)" program.  $p < 0.05$  was considered significant. Continuous variables were documented as mean  $\pm$  standard deviation and categorical variables as numbers and percentages. Mann-Whitney U test was used to compare independent group differences. Variation of follow-up data between groups was determined by ANOVA test.

## RESULTS

Fifty celiac patients who applied to adult gastroenterology clinics between December 2018-August 2019 were included in the study. The demographic characteristics of the study and control groups are shown in Table 1.

	Probiotic yogurt+GFD group (n=25) Mean $\pm$ SD	GFD group (n=25) Mean $\pm$ SD	p (<0.05)
Gender			
Female	18	18	1.000
Male	7	7	
Age	37.6 $\pm$ 12.6	34.5 $\pm$ 12	0.382
Height	164.2 $\pm$ 9.4	165.5 $\pm$ 7.8	0.582
Weight	64.4 $\pm$ 10.5	62.8 $\pm$ 12.2	0.622
BMI	23.8 $\pm$ 3.3	22.9 $\pm$ 4.5	0.425

The 0<sup>th</sup> and 3<sup>rd</sup>-month anti-tTg IgA, anti-DGP IgA and EMA values of the probiotic yogurt+GFD and GFD group are shown in Table 2. There was a statistically significant decrease in celiac markers at the end of three months in both groups ( $p < 0.001$ ).

Table 2. Changes of celiac markers in probiotic yogurt+GFD and GFD group

	Probiotic yogurt+GFD group (n=25) Mean $\pm$ SD		GFD group (n=25) Mean $\pm$ SD		p (<0.05)
	0 <sup>th</sup> month	3 <sup>rd</sup> month	0 <sup>th</sup> month	3 <sup>rd</sup> month	
Anti-tTG IgA	207.7 $\pm$ 88.7	48.1 $\pm$ 34.6	245.5 $\pm$ 101.8	83.8 $\pm$ 68.2	0.839
Anti-DGP IgA	140.7 $\pm$ 89.0	57.9 $\pm$ 48.2	146.4 $\pm$ 84.5	66.0 $\pm$ 49.0	0.854
EMA	3.4 $\pm$ 0.9	1.7 $\pm$ 1.0	3.6 $\pm$ 0.6	2.5 $\pm$ 1.1	0.025

There was no difference in ALT, AST, ALP, albumin, calcium, TSH, Hb, and platelet values between the 0<sup>th</sup> and 3<sup>rd</sup> months both in the study and control groups ( $p > 0.05$ ). When the changes in folate, vitamin B12, vitamin D, ferritin, and iron values were compared between 0 and 3 months in both groups, there was no statistically significant difference between them ( $p > 0.05$ ).

The incidence of gastrointestinal symptoms (diarrhea, constipation, abdominal pain, bloating, reflux) at 0 and 3 months in the probiotic yogurt+GFD and GD groups is shown in Table 3. There was a statistically significant decrease in the number of patients reporting clinical symptoms at the end of three months in both groups.

Table 3. Number of patients reporting gastrointestinal symptoms at 0<sup>th</sup> and 3<sup>rd</sup> months in probiotic yogurt+GFD and GFD groups

	Probiotic yogurt+GFD group (n=25)		GFD group (n=25)		p (<0.05)
	0 <sup>th</sup> month	3 <sup>rd</sup> month	0 <sup>th</sup> month	3 <sup>rd</sup> month	
Diarrhea	11	1	19	8	0.777
Constipation	14	6	12	5	0.760
Abdominal pain	19	2	15	7	0.012
Bloating	17	8	21	14	0.548
Reflux	10	2	6	1	0.338

## DISCUSSION

Relationship between a healthy life and fermented foods is still valid today, and studies on fermented products have gained momentum in recent years. There are several potential benefits of using probiotics. However, the use of probiotics as an adjunct therapy in CD has not been adequately studied. In our study, the contribution of probiotic-added yogurt to the prognosis of CD was investigated, and to the best of our knowledge, there is no such study in the literature. In this study, we evaluated the effect of regular consumption of yogurt which contains extra probiotic bacteria, on celiac markers, biochemical values, and clinical symptoms of celiac patients for three months.

In a study by Sugai E. et al. (8) antibody changes in celiac patients after GFD were examined. After three months of GFD, anti-tTG IgA decreased by 62% and anti-DGP IgA by 56%. After one year of GFD, anti-tTG IgA decreased by 73% and anti-DGP IgA by 77%. In a similar study by Jain V. et al. (9), after three months of GFD, anti-tTG IgA decreased by 52% and anti-DGP IgG by 49% in celiac patients. After six months of GFD, anti-tTG IgA decreased by 77% and anti-DGP IgG by 73%. In a study by Fotoulaki M. et al. (10), after three months of GFD, EMA decreased by 52.2% in celiac patients. In our study, there was a decrease in antibody levels at the end of three months compatible with the studies mentioned above. In the probiotic yogurt+GFD group, anti-tTG IgA decreased by 75%, anti-DGP IgA by 52% and EMA

by 53%; in the GFD group, anti-tTG IgA decreased by 63%, anti-DGP IgA by 52% and EMA by 33%. Compliance with a GFD; with or without probiotic bacteria, supplementation caused a significant decrease in celiac parameters. When the probiotic yogurt+GFD group was compared with the GFD group, it was found that the EMA test decreased significantly in the probiotic yogurt+GFD group. Although anti-tTG IgA and anti-DGP IgA levels were decreased more in the probiotic yogurt group, there was no significant difference between the two groups. Based on the studies conducted by Sugai et al. and Jain et al. (8-9), we think that if the groups in our study were able to continue their diets for a total of 6 months, the antibody values would decrease further and the difference between the groups could decrease to significant levels in all three antibodies.

Probiotics are known to prevent leaky gut, which is known to increase especially in CD, by creating a mechanical barrier that reduces the contact of gluten protein with the intestinal epithelium and strengthening tight-junctions between epithelium which at last decrease intestinal permeability. Probiotics also support GIS immunity by producing short-chain fatty acids by feeding and keeping the intestinal epithelium alive, stimulating the host's immune system and inducing the release of antimicrobial peptides (AMP), and reducing the passage of pathogenic microorganisms and allergic nutrients that may come with foods without being digested (3-7). We suggested that CD can enter the recovery process faster when consumed with a GFD via these beneficial effects of probiotics.

In a study by Håkansson Å. et al. (11), it was shown that oral administration of *L. plantarum* and *L. paracasei* significantly reduced the mean anti-tTG IgA level compared to the group receiving placebo in children who were fed a gluten-containing diet, who were not diagnosed with celiac disease but were genetically at risk of celiac disease. In a study by Smecuol E. et al. (12), the effect of *Bifidobacterium infantis* strain on the change in serological values of celiac patients who are incompatible with GFD was evaluated. Both anti-tTG IgA and anti-DGP IgA antibodies decreased by an average of 10% at the end of three weeks in the probiotic group, while anti-tTG IgA increased by 7% and anti-DGP IgA increased by 10% in the patients in the placebo group. In a study by Olivares M. et al. (13), it was investigated whether *Bifidobacterium longum* increases the efficiency of GD in celiac patients. Patients in both groups showed a decrease in celiac markers, but there was no significant difference in the decreased values between the two groups. In our study, a decrease in celiac markers was found at the end of three months in both the probiotic yogurt+GFD and GFD groups. A significant decrease was found in EMA values in the probiotic yogurt+GFD group compared to the GFD group.

Malabsorption symptoms due to decreased absorption of vitamins and minerals are common in celiac disease. In a study by Deora V. et al., vitamin values increased after GFD in patients with celiac disease (14). In addition, probiotic supplementation has also been shown to increase the amounts of iron, vitamin B12 and folate (15). In our study, a significant increase was found in vitamin D and ferritin values in the probiotic yogurt+GFD group at the end of three months. Although folate, vitamin B12 and iron values were also increased, the change between 0. and 3. months was

not significant. A significant increase was found in vitamin D, folate and iron levels in GFD group at the end of three months. Although there was an increase in vitamin B12 and ferritin values, the change between 0<sup>th</sup> and 3<sup>rd</sup> months was not significant. When the three-month changes in vitamin D, folate, vitamin B12, ferritin and iron levels of both groups were compared, no significant difference was found between them.

Irritable bowel syndrome (IBS) type symptoms can be seen in CD. It is assumed that if people with CD remove gluten from their diet, their gastrointestinal symptoms will improve. However, while the prevalence of IBS in the community was 5%, the prevalence of IBS-type symptoms was found to be 20% in celiac patients despite GFD and normalization of serum anti-tTG values. This proves that IBS-type symptoms can be permanent in celiac patients (16). Gastrointestinal system dysbiosis has also been seen in celiac patients fed with GFD. Considering that dysbiosis is associated with persistent gastrointestinal symptoms in these patients, studies have been conducted to suggest that probiotic therapy would be beneficial (17).

In a study by Martinello F. et al. (4), celiac patients and healthy subjects were given probiotic-containing yogurt for thirty days, before and after *Bifidobacterium* concentrations in stool were compared. The fecal *Bifidobacterium* concentration in healthy subjects was significantly higher than in celiac patients. After consumption of probiotic-containing yogurt, it was found that although there was a significant increase in the fecal *Bifidobacterium* concentration in both groups, the concentration in celiac patients did not even reach the concentration before consuming probiotic yogurt in healthy individuals. In a study by Smecuol E. et al. (12), the effect of *Bifidobacterium infantis* strain on the change in gastrointestinal symptoms of celiac patients without GFD was investigated. At the end of three weeks of treatment, patients in the probiotic group experienced a significant decrease in indigestion and constipation symptoms compared to the placebo group. In a study by Francavilla R. et al. (17), the effect of the probiotics on IBS symptoms of GFD compatible celiac patients with persistent IBS symptoms was investigated. At the end of six weeks, it was found that symptoms in the probiotic group decreased significantly compared to placebo. In a study by Hong K. et al. (18), the effects of probiotics on gastrointestinal symptoms were investigated in IBS patients for eight weeks. Significant reduction in abdominal pain and bloating symptoms in the probiotic group compared to placebo. In a study by Sinn DH. et al. (19), *Lactobacillus acidophilus* was found to significantly reduce abdominal pain in patients diagnosed with IBS. In the study by Rousseaux C. et al. (20), *Lactobacillus acidophilus* strain was shown to induce the expression of mu-opioid and cannabinoid receptors in intestinal epithelial cells and have analgesic functions similar to the effects of morphine in the intestine, thus shown to be effective in treating abdominal pain. In our study, a significant reduction in symptoms was found after three months in both the probiotic yogurt+GFD group and the GFD group. This confirms the important role of GFD in the treatment of symptoms of celiac patients (21). When both groups were compared, there was a significant decrease in abdominal pain symptoms in the probiotic yogurt+GFD group compared to the GFD group.



## CONCLUSION

As a result; both GFD, and GFD and probiotic yogurt consumption were found to be effective in reducing the levels of celiac markers in celiac patients. Gluten-free diet and probiotic yogurt consumption were found to have a statistically significant contribution to the decrease in EMA levels. In celiac patients, GFD only group was found to increase folate, vitamin D and iron levels; where GFD and probiotic yogurt consumption were found to increase ferritin and vitamin D levels. GFD and probiotic yogurt consumption group was found to have a statistically significant contribution to the reduction of IBS symptoms when compared with GFD only group.

Considering the results of our study, it may be suggested that when probiotic homemade yogurt is consumed together with GFD, CD may enter the recovery process faster. Besides, to our knowledge, this is the first study that showed that a natural, high-probiotic food like homemade yogurt contributes to CD recovery.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** Retrospective ethics committee approval is not required for articles which was produced from master's/doctorate thesis before 2020.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

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