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Original Article

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Can a Synbiotic Supplementation Contribute to Decreasing Anti-Tissue Transglutaminase Levels in Children with Potential Celiac Disease?

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Conflict of Interest
The authors have no financial conflicts of interest.

ABSTRACT

Purpose: Synbiotics can alleviate some intestinal pathologies or prevent trigger mechanisms for some diseases such as celiac disease (CD). If patients with high levels of anti-tissue transglutaminase (anti-tTG) immunoglobulin A (IgA) antibodies have normal duodenal histology, they are followed as potential CD patients. The aim of this study was to investigate the effect of synbiotic use on the blood levels of anti-tTG antibodies in children.
Methods: Eighty-two patients with high anti-tTG levels were included in this study. Patients were randomly divided into two groups. The synbiotic group was treated with a daily dose of a synbiotic including multi-strain probiotics for 20 days. The control group was not administered any medication. Anti-tTG values at baseline and repeat measurements and the percentage change in anti-tTG levels between groups were compared.
Results: The anti-tTG level at baseline was 36 U/mL (interquartile range [IQR], 26.4–68 U/mL) in the synbiotic group, and it decreased significantly to 13 U/mL (IQR, 6.5–27.5 U/mL) after 20 days (p<0.05). The anti-tTG level at baseline was 46 U/mL (IQR, 31–89 U/mL) in the control group, which also decreased significantly to 23 U/mL (IQR, 7–41 U/mL) after 20 days (p<0.05). Anti-tTG levels exhibited 73% and 56% decreases in the synbiotic and control groups, respectively (p<0.05).
Conclusion: It may be speculated that a synbiotic supplementation can contribute to decreasing anti-tTG levels in children with potential CD.

Keywords: Anti-tissue transglutaminase; Probiotics; Synbiotics; Celiac disease; Microbiota; Dysbiosis

INTRODUCTION

Probiotics are live bacteria that have health benefits when used in appropriate amounts. Prebiotics are non-digestible substances that enhance the effects of probiotics, and synbiotics are formulations that contain both. Probiotics play a role in maintaining the integrity of intestinal microbiota and its restoration, if it is disrupted [1,2]. Beneficial effects of probiotics on intestinal pathologies have been reported. Many studies have reported that pre-, pro-, and synbiotics prevent, treat, or alleviate intestinal pathologies such as celiac

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disease (CD), inflammatory bowel disease, and irritable bowel syndrome by balancing the microbiota and regulating immune responses [1,2].

The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition defines CD as an immune-mediated systemic disorder induced by gluten and related prolamins in genetically susceptible individuals with human leukocyte antigen (HLA)-DQ2 or HLA-DQ8 haplotypes [3]. Anti-tissue transglutaminase (anti-tTG) immunoglobulin A (IgA) autoantibody plays a central role in the pathogenesis of CD, and it is one of the specific markers used in diagnosing the disease. In CD, disruption of the intestinal barrier increases anti-tTG levels, which in turn exacerbates the disruption. Anti-tTG levels are directly proportional to the severity of intestinal damage. To confirm a diagnosis of CD in patients with high anti-tTG levels, endoscopic duodenal biopsy specimens should be histopathologically evaluated, except for some limited conditions. Patients who are not diagnosed with CD after histopathological evaluation are considered as potential CD patients. Patients who are diagnosed with CD are started on a gluten-free diet, and elevated anti-tTG levels return to the normal range within a short period of time [3-5].

The present study aimed to investigate the effect of probiotics/synbiotics on the levels of anti-tTG IgA antibodies. Therefore, patients with high anti-tTG levels but who were not histopathologically diagnosed with CD were included in the study group. The purpose was to prevent these patients from receiving another therapy as patients diagnosed with CD would need to start a gluten-free diet, after which anti-tTG levels would return to normal. Consequently, no information would be obtained about the progress of anti-tTG levels with only synbiotic therapy or without any therapy. It would also be unethical to begin synbiotic treatment before beginning a gluten-free diet in patients diagnosed with CD. To the best of our knowledge, this study is the first to investigate the positive effect of synbiotics use on anti-tTG levels.

MATERIALS AND METHODS

Patients

Patients with chronic gastrointestinal disorders (such as diarrhea, constipation, gastroesophageal reflux, dyspepsia, and abdominal pain), growth retardation or isolated short stature, type 1 diabetes mellitus, iron deficiency anemia, alopecia, and clubbing were tested for anti-tTG IgA antibodies. Eighty-two patients with high anti-tTG levels assayed after suspicion of CD and who subsequently underwent duodenal biopsy to confirm CD but were not diagnosed with CD after histopathological evaluation were included in this study. Patients diagnosed with CD, giardiasis, *Helicobacter pylori* infection, cow's milk protein allergy, inflammatory bowel disease, and those receiving drug treatment for any other disease were excluded from the study.

Patients were randomly divided into either a synbiotic or a control group, each comprising 41 patients. Patients in the synbiotic group were treated with a daily dose of a synbiotic including multi-strain probiotics (NBL Probiotic Gold cachet; Nobel, Istanbul, Turkey; including 2.5×10^9 cfu live bacteria including *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, 625 mg fructooligosaccharide, and vitamins A, B1, B2, and B6) for 20 days. Patients in the control group were not administered any medication. Anti-tTG levels were re-measured in all patients after 2–6 months.

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This study was approved by the institutional non-invasive clinical research ethics committee of Yuzuncu Yil University (2014/04). Informed consent was obtained. Patient information was kept confidential, and the study was conducted according to the tenets of Declaration of Helsinki.

Anti-tissue transglutaminase IgA antibody assay

Anti-tTG IgA antibodies were evaluated by a human recombinant enzyme-linked immunosorbent assay, using a commercially available kit (AESKULISA tTG; Aesku Diagnostics, Wendelsheim, Germany).

Statistical analysis

The statistical review of the study was performed by a biomedical statistician. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Co., Armonk, NY, USA). Data showing non-Gaussian distributions were presented as medians with interquartile ranges (IQRs). The distributions of the baseline and repeat anti-tTG levels of the groups were checked for normality using the Shapiro-Wilk test, and the comparisons of the intra-group values were performed using the sign test. To calculate the percent change of anti-tTG levels in each group, the following formula was used: [100−(2nd anti-tTG value×100/1st anti-tTG value)]/100. The two independent groups were then compared using the Mann-Whitney U-test. Pearson's chi-squared test was used when comparing categorical variables. A *p*-values <0.05 were considered statistically significant.

RESULTS

The median age of the patients was 7 years (IQR, 4–12 years) and 51% were female. **Table 1** provides details regarding age and sex distributions of the groups, **Table 2** anti-tTG values at baseline and repeat measurements within groups, and **Table 3** the percentage change in anti-tTG levels at baseline and repeat measurements between the groups.

The anti-tTG level at baseline was 36 U/mL (IQR, 26.4–68 U/mL) in the synbiotic group, and it decreased significantly to 13 U/mL (IQR, 6.5–27.5 U/mL) following synbiotic use ($p<0.05$). The anti-tTG level at baseline was 46 U/mL (IQR, 31–89 U/mL) in the control group, which also decreased significantly to 23 U/mL (IQR, 7–41 U/mL) although these patients did not undergo any therapy ($p<0.05$). According to the percent change calculated to understand which group

Table 1. Age and sex distribution of the groups

Groups	Age (yr)	Sex (female/male)
Synbiotic group	6 (3.5–11)	21/20
Control group	9 (4–13.5)	21/20
p-value	0.11*	1†

Values are presented as median (interquartile range) or number only.
*Mann-Whitney U-test. †Pearson's chi-squared test.

Table 2. Anti-tTG values at baseline and repeat measurements within the groups

Groups	Baseline anti-tTG	Repeat anti-tTG	*p*-value
Synbiotic group	36 (26.4–68)	13 (6.5–27.5)	0.001*
Control group	46 (31–89)	23 (7–41)	0.001*

Values are presented as median (interquartile range).
Anti-tTG: anti-tissue transglutaminase.
*Sign test.

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Table 3. Percentage change in Anti-tTG levels at baseline and repeat measurements between the groups

Groups	% change
Synbiotic group	0.73 (0.56–0.83)
Control group	0.56 (0.32–0.76)
p-value	0.04*

Values are presented as median (interquartile range).
Anti-tTG: anti-tissue transglutaminase.
*Mann-Whitney U-test.

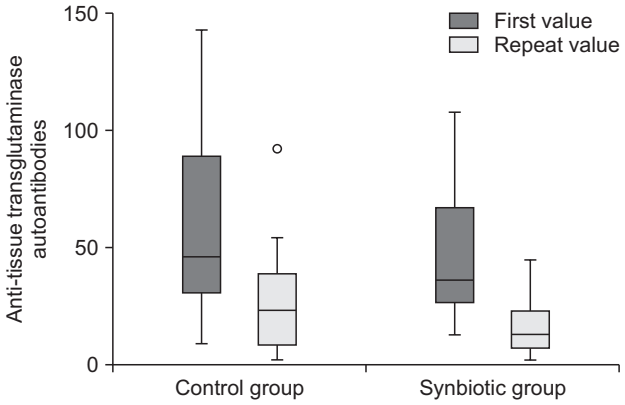

Fig. 1. Comparison of the percentage change in anti-tissue transglutaminase levels at baseline and repeat measurements between groups.

showed a higher decrease, anti-tTG levels exhibited 73% and 56% decreases in the synbiotic and control groups, respectively (**Fig. 1**). There was a significant difference between the two groups (p<0.05).

DISCUSSION

CD results from the interplay of genetic, environmental, and immunologic factors. The strongest and best-characterized genetic susceptibility factors in CD are the HLA-DQ2 and HLA-DQ8 haplotypes. Environmental factors clearly play an important role in the pathogenesis of CD. The primary trigger in the disease is gluten. Gluten cannot be completely digested even in the intestines of a healthy person, and it contains toxic gliadin proteins that are made up of glutamine and prolamin residues. The HLA-DQ2 and HLA-DQ8 variant proteins are found on the surface of intestinal antigen-presenting cells. The undigested gliadin peptides present in the intestinal lumen can cross the intestinal epithelium via transcellular transport or paracellular transport; the latter occurs if the intestinal barrier is disrupted. These peptides are deaminated with tTG in the lamina propria and undergo cross-linking. Once the glutamine contained in gliadin is converted to glutamic acid, it binds to HLA-DQ2 and HLA-DQ8, and it is presented to CD4+ T cells. Gliadin-tTG crosslinks lead to the formation of tTG antibodies, which inhibit the function of tTG. Activated gluten-reactive CD4+ T cells produce high levels of pro-inflammatory cytokines. These cytokines also involve T-helper cells into the process and the inflammatory effect is further aggravated. Matrix metalloproteinases secreted from the lamina propria and fibroblasts lead to the breakdown of extracellular matrix and damage the basement membranes. This results in an increased level of lymphocytes and natural killer T cells within the epithelial cell. Gliadins also upregulate the expression of zonulin protein, increasing intestinal permeability in both

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CD patients and healthy individuals. Moreover, increased anti-tTG levels inhibit tTG and complicate the digestion of gliadin. This in turn increases tTG activity and produces a vicious cycle. Consequently, characteristic lesions of CD develop by apoptosis [4,6-8]. CD resembles other autoimmune diseases, but it differs by the presence of a trigger: namely gluten. If gluten is not present, intestinal lesions recover and autoantibodies decrease. Anti-tTG antibodies can emerge and induce CD before villous atrophy develops. Although anti-tTG antibodies actually emerge as an epiphenomenon in protection against the disease, they play a central role in the pathogenesis of the disease [7].

The goal has always been to prevent the manifestation of CD in patients at high risk of developing CD (those with a first-degree relative with CD and HLA DQ2- or -8-positive individuals) or individuals monitored as potential CD patients. Vriezinga et al. [9] introduced gluten in 4- to 6-month-old infants at high risk of developing CD in order to prevent the development of CD but could not achieve a reduction in the disease development during a 3-year follow-up. Borelli et al. [10] asserted in their study that serum and intestinal anti-tTG levels were correlated in patients with suspected CD, even though they were not diagnosed with CD, and that intestinal anti-tTG levels had a predictive value for villous atrophy. Therefore, lowering tTG and thereby anti-tTG levels has been a therapeutic goal in CD, and there is an increasing number of preclinical studies on this subject [11]. The present study contributes to this aim focusing on anti-tTG levels.

Dysbiosis is defined as a lack of mutualistic relationships among bacteria and a decrease in the number of beneficial bacteria (especially lactobacilli and/or bifidobacteria). Intestinal dysbiosis has been implicated in the pathogenesis of many diseases, such as CD, inflammatory bowel disease, and irritable bowel syndrome. Therefore, restoring intestinal microbiota is a therapeutic goal. Most commonly, probiotics, prebiotics, and synbiotics are used for this purpose [2]. The synbiotic given to the patients in the present study contained *Lactobacillus species* and *Bifidobacterium species*.

Intestinal dysbiosis has been commonly reported in patients with CD. Although there are various studies on the disease, the decrease in the ratio of Gram-positive/Gram-negative bacteria in CD patients compared with that in healthy individuals has drawn attention, especially the decrease in the number of *Bifidobacteria* and the increase in the number of virulent Gram-negative *Bacteroides* [12-14]. It has been reported that the number of Gram-negative bacteria (*Bacteroides* spp., *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Neisseria* spp., and *Prevotella* spp.) isolated from CD patients had increased and that pathogenic Gram-positive bacteria (*Clostridium* spp., *Staphylococcus* spp., and *Actinomyces* spp.) could also be isolated from CD patients. In addition, HLA genotypes contribute to the development of CD by affecting gut microbiota [15]. Consequently, it was asserted that dysbiosis has a primary or secondary role in the pathogenesis of CD.

It has been reported that intestinal dysbiosis plays a role in both triggering and inducing CD and that it aggravates CD in patients even if they are on a gluten-free diet [16]. In a study by Galipeau et al. [17], intestinal microbiota models of mice expressing the human DQ8 molecule showed that gluten-induced immunopathology had both a positive and negative correlation with the models. They also asserted that intestinal microbiota changes could increase the risk of CD in genetically susceptible individuals; therefore, specific microbiota-based treatments could be helpful in preventing or treating CD.

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In both animal and human studies, *Bifidobacterium* spp. [18,19] and *Lactobacillus* spp. [20] reversed the harmful effects of gliadin on the epithelium. In a study by De Angelis et al. [21], it was claimed that VSL#3 (including *Lactobacillus* and *Bifidobacterium* strains), a multi-strain probiotic, facilitated gliadin digestion and tolerability due to its proteolytic effect; therefore, it could remove traces of toxic peptides in processed foods and provide a better taste to gluten-free products. However, Harnett et al. [22] reported that they did not observe a significant change in the number of microorganisms in gastrointestinal microbiota of adult CD patients who received VSL#3. Francavilla et al. [23] indicated that they reduced the severity of symptoms of irritable bowel syndrome in CD patients who adhered to a gluten-free diet by increasing the number of *Bifidobacteria* in intestinal microbiota. In a study by De Palma et al. [24], it was shown that a decreased number of *Bifidobacteria* and an increased number of pathogenic Gram-negative bacteria increased Th1-type pro-inflammatory cytokine levels and also contributed to monocyte maturation and T-cell increases in CD patients. In the present study, the synbiotic including *Lactobacillus* and *Bifidobacterium* strains may impact anti-tTG levels with such mechanisms.

The limitation of the study was the lack of detection of HLA-DQ2 and/or HLA-DQ8. However, HLA testing is not required for a serology-based diagnosis without biopsies for diagnosis of CD according to the current guideline [25]. In addition, although the patients' repeat anti-tTG levels were planned to be examined after 2 months, some patients' admission was delayed to after 6 months. This may have affected the results. However, since the study was observational and the patients were selected randomly, the results were evaluated as such.

In conclusion, anti-tTG levels decreased significantly in the synbiotic group compared with that in the control group. This decrease in the synbiotic group was significantly higher than that in the control group. Patients with high anti-tTG levels, in whom histopathological evaluation has not confirmed the diagnosis of CD, may consider using synbiotics as they may decrease anti-tTG levels as shown in our study. The long-term effects have not been studied in our study.

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