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The effects of provocation by foods with raised IgG antibodies and additives on the course of Crohn's disease: A pilot study

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Background/aims: *This study was designed to assess the role of foods with raised IgG antibodies and additives on the symptoms and inflammation of Crohn's disease.* ***Methods:*** *Eight patients with Crohn's disease in remission were studied. They followed a strict diet during phase I. Then, provocations with two, three-day periods (phases II and III) followed: in phase II, pure forms of foods with high IgG antibodies and in phase III, off-the-shelf forms of those foods were added. Stool samples were collected for fecal calprotectin assay. Blood samples were taken on the 11th and 17th days for highly sensitive C-reactive protein, ferritin, erythrocyte sedimentation rate, white blood cells, and platelets. Patients kept a diet-symptom diary.* ***Results:*** *Increased Crohn's disease activity index scores were found statistically significant (p=0.012) between pre- and during the provocation weeks. There were significant increases according to Harvey-Bradshaw Index when the highest values during the phases I, II (p=0.027) and I, III (p=0.027) were compared. The increases in highly sensitive C-reactive protein (p=0.025) and white blood cells (p= 0.036) were found statistically significant. Fecal calprotectin levels showed day-to-day variability. When compared, the levels of fecal calprotectin increased in all patients on the last day of the restriction (10th day) and the first day of the provocation (11th day) with the exception of one patient.* ***Conclusions:*** *Foods with raised IgG antibody levels and food additives can provoke the symptoms and may stimulate the inflammation in patients with Crohn's disease. Addition of a proper diet with restriction of those foods may be beneficial in the medical treatment.*

Key words: Crohn's disease, food-specific IgG antibodies, activity indexes, acute phase response, fecal calprotectin

Yüksek IgG antikoru olan gıdalarla ve katkılarla yapılan provokasyonun Crohn hastalığının seyri üzerine etkileri: Ön çalışma

Amaç: *Bu çalışma yüksek IgG antikorları olan gıdaların ve katkıların Crohn hastalığında semptomlar ve inflamasyon üzerine olan etkilerini değerlendirmek amacıyla planlanmıştır.* ***Yöntem:*** *Çalışmaya remisyonda olan 8 Crohn hastalığı olgusu alındı. Hastalar faz I süresince sıkı diyet uyguladılar. Daha sonra üçer gün süreli 2 provokasyon uygulandı (faz II ve III); faz II'de yiyeceklerine yüksek IgG'si olan gıdaların saf şekilleri, faz III'de de bu gıdaların hazır şekilleri eklendi. Fekal kalprotektin incelemesi için gaita örnekleri toplandı. Lökosit, platelet, sedimantasyon, hassas-C-reaktif protein ve ferritin için kan örnekleri 11. ve 17.gün alındı. Hastalar diyet-semptom günlüğü tuttular.* ***Bulgular:*** *Provokasyon öncesi ve sırasındaki haftalara ait Crohn hastalığı aktivite indeksi skorları arasında anlamlı artış bulundu (p=0.012). Harvey Bradshaw indeksine göre, faz I, II (p=0.027) ve I, III (0.027) dönemlerindeki en yüksek değerler karşılaştırıldığında, anlamlı artışlar bulundu. Lökosit (p= 0.036) ve hassas-C-reaktif protein (p=0.025) değerlerindeki artış istatistiksel olarak anlamlı bulundu. Fekal kalprotektin düzeyleri günlük değişiklikler gösterdiler. Fekal kalprotektin düzeyleri kısıtlamanın son günü (10. gün) ile provokasyonun ilk günü 11. gün) karşılaştırıldığında 1 hasta dışında hepsinde artış gösterdi.* ***Sonuç:*** *IgG seviyeleri yüksek olan gıdalar ve gıda katkıları Crohn hastalığı olan olgularda semptomları provoke edebilir ve inflamasyonu stimule edebilir. Bunların kısıtlanması ile hazırlanan diyetin eklenmesi tıbbi tedaviye yardımcı olabilir.*

Anahtar kelimeler: Crohn hastalığı, gıda spesifik IgG antikorları, aktivite indeksleri, akut faz yanıtı, fekal kalprotektin

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INTRODUCTION

According to the current consensus, genetic, immunological and environmental factors such as enteric microflora and nutritional environment have been accepted as playing a role in the pathogenesis of inflammatory bowel disease (IBD) (1). Although luminal flora is the most emphasized factor, the nutritional environment is also important since large amounts of food pass through the gastrointestinal tract each day. Disease flares occur in random ways; they may manifest in very short durations, i.e. within 1-2 days, and are often unpredictable. Many patients feel that their symptoms are often exacerbated when they eat certain foods.

It is known that the elemental diet is as effective as corticosteroid treatment in achieving remission in patients with Crohn's disease (CD) for a short duration; however, relapses are likely when patients resume their normal diets (2,3). Some beneficial effects such as longer remission periods were reported with another diet modality called the exclusion diet, where a new food is introduced each day and excluded if the food causes any symptoms (4-6). It should be noted that the exclusion diet is not universally accepted. Furthermore, there are no specific dietary approaches to the management of CD. Dietary advice to these patients is usually a nutritionally balanced diet without restrictions unless they experience intolerance to specific foods.

Recently, some studies have demonstrated that food elimination, based on raised IgG antibodies, may be effective in reducing symptoms in patients with irritable bowel syndrome (IBS) and CD (7-10). We have been using food-specific IgG test in some patients with CD, ulcerative colitis (UC) and functional dyspepsia overlap with irritable bowel syndrome (FD-IBS). We have observed symptomatic improvements in the majority of these patients when they have followed a strictly controlled diet.

The altering course of IBD may cause many problems and questions in the long-term food-related studies. Unexplained long duration of remission periods occasionally occur in CD. It was reported that 15% of patients with CD were symptom-free for two years when treated with placebo alone (11). Many factors, such as super infections, antibiotic treatment and ongoing usage of immune suppressive drugs may affect the outcome of the studies. The most important one is possibly the lack of patient compliance with food elimination in the long-term application. Hence, we planned our study to include a short-term provocation. In this study, we evaluated the symptoms and also added objective measurements like white blood cells (WBC), platelets (PLT), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and ferritin as acute phase markers, and fecal calprotectin (FC) as the intestinal inflammation marker.

C-reactive protein (CRP), which is an objective and sensitive marker of inflammation, correlates well with disease activity, endoscopic findings and severity of active histological inflammation in CD patients (12). CRP rises rapidly in response to acute phase stimulus and also decreases rapidly once the stimulus disappears because of its short biological half-life of 19 hours (13).

Recently, FC has been used as a biomarker of intestinal inflammation. Calprotectin is a 36.5 kDA calcium- and zinc-binding protein derived predominantly from neutrophils (14,15). It was reported that FC might reflect the migration of granulocytes through the gut wall in patients with IBD by using indium-111-labeled granulocytes (16).

To our knowledge, this pilot study is the first investigation to show the effectiveness of provocation of foods with high IgG levels on the clinical course of CD. Unfortunately, many food additives are used extensively in our daily food stuff, and the IgG test that we have used includes only a few of these additives. Hence, we designed a two-step provocation study with only the pure form of foods taken in the first step and foods containing additives taken during the second step.

MATERIALS AND METHODS

Patients

Eight CD patients (7 female, 1 male; mean age: 40±8.16) who had been in remission were studied. These patients were selected among 22 CD patients who were tested earlier for food- specific IgG antibody, and they had been following the elimination diet based on IgG antibodies for a period of 6-30 months. They were following the dietary instructions regularly except on some occasions.

Diagnosis of CD was based on the clinical, endoscopic, radiological, and histopathological criteria. Remission was determined by the disease activity assessment, according to the Crohn's disease activity index (CDAI) of Best et al. (17). Clinical features and characteristics of their disease are shown in Table 1.

Table 1. Patients' characteristics

No	Age/ Gender	Disease age (year)	Previous operation(s)	Disease location	Fistula	EIM	Smoking
1	48 F	7	-	C	Perianal	U, EN	-
2	42 F	22	Twice *	I+TI+C	Perianal	R	+
3	45 M	3	-	TI+C	-	-	-
4	31 F	17	Twice **	I+TI+C	-	R, EN	-
5	44 F	8	-	TI+C	Perianal	-	+
6	48 F	14	Once ***	TI+C	Ileovesical	-	-
7	26 F	1	Once ****	TI+C	-	-	-
8	36 F	5	-	TI+C	-	-	+

* 1st: Terminal ileal resection and right hemicolectomy due to stenosis.

2nd: Transverse and left colon resection due to stenosis of distal left colon; polypoid mass (2 cm in diameter) in the transverse colon could not be reached by colonoscope.

**1st: Laparotomy for massive bleeding.

2nd: Terminal ileal resection and right hemicolectomy due to stenosis.

***Terminal ileal resection and right hemicolectomy due to ileovesical fistula.

****Terminal ileal resection and right hemicolectomy due to stenosis.

C: Colonic. TI: Terminal ileal. I: Ileal (involvement areas cover whole disease period). EIM: Extraintestinal manifestations (during whole period of disease). U: Uveitis. EN: Erythema nodosum. R: Rheumatologic manifestations (ankylosing spondylitis, no: 2; sacroiliitis + peripheral arthritis, no: 4).

The patients who were accepted to this study had not taken any medication (except 5-aminosalicylic acid [ASA]) for at least six months or antibiotics for a period of two months prior to the study. In order to increase the reliability of the study, the patients chosen for this study had also agreed to prepare all of their foods at home, paying careful attention to the details of the study.

Patients treated with drugs such as azathioprine, 6-mercaptopurine or anti-tumor necrosis factor (TNF) within the past 12 months and glucocorticoids within six months were excluded from the study. Patients with high serum IgE levels were also excluded.

The study protocol was approved by the Ethics Committee of Cerrahpaşa Medical School, İstanbul University, and written informed consents were obtained from all participants.

Study Design

All patients were asked to stop taking supplements such as calcium, cobalamine and folic acid one month prior to the study. They continued to receive 5-ASA at the same dosage throughout the study period.

Food-specific IgG antibodies were examined by an enzyme-linked immunosorbent assay (ELISA) to a panel of 266 different food and additive antigens. According to the manufacturer's recommendations (ImuPro 300 test; Evomed/R-Biopharm AG, Darmstadt, Germany), levels of 7.5 µg/ml or above are accepted as a positive.

The patients were instructed to follow a strict diet during the first 10 days (phase I) to exclude the foods with positive IgG antibody levels and additives. Phase I was followed by a provocative period lasting for a total of six days, consisting of two "three-day" periods (phases II and III). Phase II (11th -13th days) was the first stage of the provocative period. A maximum of three foods without additives with the highest food-specific IgG antibody levels were added to the diets on the first provocation day and foods were changed according to the IgG antibody levels list on the following days. In phase III (14th -16th days), additives were added to the diet followed in phase II. This was accomplished by using off-the-shelf packaged foods and ingredients in the cooking of the patients' foods. Their foods such as bread, pasta, yogurt, and tomato paste were purchased from the market instead of making them at home.

Personalized diet plans were prepared for each patient. Since starch constitutes an important part of their diet, this topic needed special attention. According to IgG antibody level results, in patients with positive wheat IgG antibody, wheat flour in their diet was replaced with corn flour or homemade rice flour. Corn flour was obtained from rural villages to reduce any chance of contamination of additives. Patients who were allowed to eat

wheat during phases I and II were instructed to use organic wheat flour.

The patients were not allowed to drink milk, alcohol or any beverages such as cola or commercial fruit juices (only water and tea) during the entire period of this study. Filtered coffee, coffee cream and cube sugar were also excluded from their diet.

Collection and Analysis of Stool Samples

The patients were instructed to collect samples of their first stool of the day during the last four days of the restriction period and every day of the provocation periods. Provocations were performed after the stools belonging to the 10th and 13th days were taken.

The samples were frozen at -20°C within 15 minutes in the patient's own home refrigerator at first, and then they were stored in the hospital at -80°C until the day of analysis. FC was measured using FC kits, commercially available quantitative Bühlmann ELISA kits® by Bühlmann Laboratories (Schönenbuch, Switzerland). Micro ELISA assay was performed in a blinded fashion. All samples were coded and assayed in duplicate.

Diet-Symptom Diary

During the study including the 17th day, patients kept a diet-symptom diary, recording their symptoms and what they ate. They marked the times, duration and severity of the symptoms. Scores of severity of abdominal pain (0-3) and their general well-being (0-4) were noted according to CDAI (17) and Harvey Bradshaw (H&B) (18) activity index as well as the defecation counts, with the characteristics of each stool as normal, loose, very soft, and liquid. They also noted the presence of abdominal distention, nausea and non-gastrointestinal symptoms such as headache or bone and joint pain.

Blood Samples

Blood samples were taken on the 11th and 17th days after a 12-hour fast to measure complete blood count, ESR, CRP, and ferritin. Highly-sensitive assay (hsCRP) was used for the detection of CRP.

Clinical Activity Indexes

Crohn's disease activity indexes (CDAIs) were calculated according to the results of seven days of the pre-provocation period and of the provocation days, including the results of the 17th day. H&B indexes were calculated each day for 16 days.

Statistical Analyses

The analyses were carried out using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL) software version 10.0. Wilcoxon signed-rank tests were utilized for statistical analyses.

RESULTS

The mean number of positive IgG antibodies against food antigens and food additives was 26.0±25.4 (range: 5-80, median: 30). Of these 8 patients, 5 had high antibodies against wheat, 5 against yeast, 3 against cow's milk and dairy products, and another 3 against eggs. Positive IgG antibodies against two different food additives (agar and guar gum) were observed in 7 patients.

One of the patients (patient 7) made the first provocation only on the 11th day. She had to stop the provocation because of severe abdominal pain. The second provocation on the 14th day caused her to stop again for the same reason. Patient 8 also stopped the provocation before the final day due to severe abdominal pain. The blood samples were taken from these two patients on the 15th and 16th days.

Abdominal pain and distention, deterioration of general well-being, nausea, and an increase in stool frequency and liquidity were observed in patients during the provocative periods (Table 2).

The mean CDAI score before the provocation was 63±29.9 (range: 26-121) and increased to 99.75±46.1 (46-183) during provocation, and this increase was found to be statistically significant (p=0.012). The daily data of patient symptoms were calculated in terms of H&B, and reached 5 in 2 patients (Table 3). When the highest H&B index

Table 2. Number of patients with symptoms in each period and mean defecation count

Symptoms	Phase I	Phase II	Phase III
Nausea	0	3	1
Abdominal distention	1	6	3
Abdominal pain	0	4	5
Deterioration in well-being	0	3	5
Headache	0	1	1
Joint and bone pain	0	0	1
Defecation count/day	1.3	1.7	2.7

Phase I: strict restriction of foods, with raised IgG antibodies and additives; Phase II: pure food provocation based on raised IgG antibodies; Phase III: provocation by foods based on raised IgG antibodies and additives.

Table 3. The highest values of each period according to H&B index

Pt. No.	Phase I	Phase II	Phase III
1	1	1	1
2	0	1	5
3	0	1	1
4	0	0	1
5	0	3	2
6	2	4	2
7	0	3	3
8	1	5	4

Phase I: strict restriction of foods, with raised IgG antibodies and additives; Phase II: pure food provocation based on raised IgG antibodies; Phase III: provocation by foods with raised IgG antibodies and additives.

scores in the phases were compared, there were significant increases between phase I (0.50±0.76) and phase II (2.25±1.75) (p=0.027) and between phase I and phase III (2.38±1.51) (p=0.027).

In one of the patients with fistula (patient 1), the increase in secretion was obvious. In another patient (patient 2), while there was only slight secretion in the beginning (Figure 1), edema and pain occurred after phase III (Figure 2). This patient's pain disappeared and her fistula returned to its original appearance (Figure 3) 15 days after she returned to her diet. The fistula in the third patient (patient 5) was inactive for 3 months, and stayed inactive at the end of phase III (Figure 4). Since this patient had been under food elimination for a long time, she decided not to return to her original diet restriction because of no change in fistula in either case. Eleven days after the beginning of the provocation phase, she had pain, edema, hyperemia, and secretion in the fistula region (Figure 5). Eleven days after she returned to her food restriction, the fistula ceased and returned to its original appearance without administration of any antibiotics (Figure 6).

Increases in WBC and hCRP levels before and after provocation were found to be statistically significant. The mean WBC value was 6980±1900, and increased to 8390±2320 mm^3, p=0.036. The mean hCRP value increased from 15±27.6 to 17.15±27.8 mg/L, p=0.025. The changes in PLT (277130±56920 vs 287630±52710 mm^3, p=0.32), ESR (22.9±17.2 vs 23.4±18.4 mm/h, p=0.23) and ferritin (66.5±845 vs 70.1±76.61 ng/mL, p=0.89) were not statistically significant.

Fecal calprotectin (FC) concentrations (n<50 µg/g) showed high values and daily changes even in

Figure 1. Appearance of the anal fistulas of the patient (No. 2) before provocation.

Figure 2. Appearance of the anal fistulas of the same patient (No. 2) after provocation.

Figure 3. Appearance of the same patient (No. 2) 15 days after returning to diet.

Figure 4. Appearance of the anal fistula of patient No. 5 before provocation.

Figure 5. Appearance of the same patient (No. 5) on the 11th day, while still under the provocation.

Figure 6. Appearance of the same patient (No. 5) 11 days after returning to diet.

days of restriction in 6 patients. When FC concentrations on the final day of restriction (10th day) and the first day of pure IgG-positive food provocation (11th day) were compared, increases were found in all patients except one who had included an additive, i.e., citric acid, to the homemade jam that she consumed in restriction days.

When this patient's (patient 2) FC values were excluded, a statistically significant increase in values was obtained between stool FC levels on the 10th and 11th days in 7 patients: 337±389.2, 713.7±702 µg/g, respectively (p=0.018) (Table 4). Evaluations achieved in a total of 7 patients, excluding patient 2, taken from samples on the 2nd day (12th day) of phase II (pure IgG-positive food provocation) and on the 2nd day (15th day) of phase III (in which food additives were included), were found statistically significant: 399.6±377.5, 687.1±643.1 µg/g, respectively (p=0.043). Furthermore, when the FC values were compared, there were statistically significant increases between the 9th (368.57±321.96 µg/g) and 11th (p=0.028); 9th and 15th (p=0.028); and 10th and 15th (p=0.028) days.

DISCUSSION

In this study, the effects of the foods with high IgG antibody levels and additives on the symptoms and inflammation in CD were studied in two provocation periods, each of three days' duration. CDAI and H&B index showed significant increases with the provocation. The statistically significant increases found in comparison of WBC and hCRP values of samples taken at the beginning and at the end of the provocation periods were the objective data, supporting the role of foods and additives on the inflammation of CD. Since the CDAI and the blood tests included both of the provocation periods, it is difficult to make any comments as whether these changes are related purely to foods or to both foods and additives. However, the statistically significant increase, found in the comparison of the highest H&B indexes in two phases (the restriction and the first provocation periods), may be evidence that the foods with high IgG antibodies provoke some symptoms of CD.

During the restriction period, the value of FC in most of the patients was found higher than normal limits and showed daily variations. The FC values of CD patients in remission were generally found to be higher than normal limits and this is considered to be due to the presence of low-grade inflammation (15,19,20). Day-to-day variations were

Table 4. Fecal calprotectin concentrations in stool samples on 10th and 11th days (μg/g)

Pt. No.	10th day	11th day
1	1020	1300
2	1310	625
3	16	52
4	740	1950
5	135	270
6	255	660
7	180	720
8	13	45

reported by Huseybe et al. (21), even in healthy volunteers. The authors suggested the need for further studies in terms of both origin and pattern of FC. Except for one patient, observation of FC increases in stool samples taken on the last day of restriction and the 1st day of provocation, which were the most marked day-to-day variations, suggests that the diet might be one of the factors having a role in FC changes. Studies that relate diet and FC values are limited, and paradoxical results were obtained when formula-fed and breast-fed infants were compared (22-24).

The dietary microparticles such as titanium dioxide, e.g. E171, which is used for whitening and brightening food, or aluminosilicates (e.g., E554-6 and E559), which are used as anti-caking agents for granular and powdered food products, are consumed very widely in western foodstuffs (25). The beneficial effect of a low microparticle diet in ileal CD was reported (25). It was shown that these microparticles are not immunologically inert, and in the presence of the bacterial lipopolysaccharides, they could act as adjuvants to induce potent cytokine responses in IBD patients (26). In our study, the patients were not restricted from using tap water, salt, powdered sugar, cheese, and toothpaste, which contain microparticles. These particles and possible undetected additives may also play some role in the day-to-day variations in FC.

Any explanation regarding the presence of high IgG antibodies against some foods and the decrease in symptoms after their elimination in patients with IBS remains speculative. Some hypotheses have been suggested in patients with IBS, such as selective permeability to food antigens (27,28) and different antigenicity of various foods (27). Development of high antibody titers for different foods could not be explained by normal physiologic response of the gut immune system because of increased permeability (27,28). If this was related to increased permeability, high antibody titers against all foods, especially to the constantly consumed foods, should be expected. However, in our unpublished series, while the rates of patients with raised IgG antibodies against wheat were found to be 65.5%, 40% and 41.9% in CD, UC and FD-IBS, respectively, the rates in patients with raised IgG antibodies against potatoes and olives, which are commonly consumed in our country, were found to be 3.8%, 4% and 0% for the same three diseases, respectively. These results can be related to the different antigenicity of the foods. Furthermore, the percentage of patients in whom high antibody titers against yeast, another substance that exists in bread besides wheat, was 61.5%, 8% and 32.3% in CD, UC and FD-IBS, respectively. This may be explained by the presence of selective permeability against some foods in different diseases. Furthermore, it has been suggested that the antibodies could be the results of different immune responses from specific food antigens (28) and genetic factors that may influence these types of reactions in different disorders.

Food antigens that may contribute to gut inflammation were shown by Van Den Bogaerde et al. (29). The authors used six food antigens and found significantly more common proliferative response in CD patients than in controls with peripheral blood lymphocyte proliferation studies. In the *in-vivo* part of the study, they measured the rectal mucosal blood flux with Doppler blood flowmetry immediately after antigen exposure and found significant increased blood flux to yeast and citrus fruits sites. Increased lymphocyte proliferation response to baker's yeast was also reported in another study in patients with CD (30). Barclay et al. (31) reported results suggesting that dietary yeast might affect the activity of CD.

The best study related with the effect of foods in CD was reported by Jones et al. (4). They reported that 51 out of 77 patients remained well on the exclusion diet alone for periods up to 51 months, with an average annual relapse rate of less than 10%. A similar result was reported by a multicentre trial (5). The median length of remission was found to be 7.5 months in the exclusion diet group, whereas it was 3.8 months in the prednisolone treatment group. In this study, food intolerances were discovered predominantly to wheat, yeast and dairy products. In the study of Jones et al. (4), symptom-

producing foods such as wheat, dairy products and yeast were ranked 1st, 2nd and 5th, respectively. In our study, the most common positive IgG antibodies were found against wheat, yeast and agar.

Food elimination is not easy to follow, even though the number of foods that need to be eliminated is limited. Female patients cook the meals by themselves and are more successful in continuing with the diet; therefore, the study group was mainly composed of female patients, with the exception of one male.

In conclusion, consuming foods with raised IgG antibody levels and additives might have some influence on the clinical outcome and inflammation of CD, since provocation with them deteriorates the clinical table and increases some of the inflammatory markers. IgG antibodies seem to help patients in identifying the candidate foods for elimination. Addition of a proper diet with restriction of these foods and additives may be beneficial in the medical treatment. Our results are open to further investigations.

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