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ORIGINAL ARTICLE

Treatment of Crohn's Disease with an IgG4-Guided Exclusion Diet: A Randomized Controlled Trial

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Abstract
Background and Aim We previously reported an improvement in symptoms in Crohn's disease following an IgG4-guided exclusion diet in an open-label study. We aimed to evaluate, in a double-blinded randomized sham-controlled setting, the efficacy of IgG4-guided diet in improving quality of life in patients with Crohn's disease.
Methods Consecutive patients with Crohn's disease and a Crohn's disease activity index (CDAI) of 80–400 attending tertiary and secondary care centers were screened. All patients had IgG4 titers tested against 16 common food types using ELISA. The true diet group excluded four food types with the highest antibody titers for 4 weeks, and the sham group excluded four foods with the lowest antibody titers. Quality of life was assessed using the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) at beginning and end of the trial. Secondary outcome measures were CDAI, Harvey Bradshaw index, serum C-reactive protein, and fecal calprotectin.
Results One hundred and forty-five subjects were screened and 96 subjects had initial food antibody testing performed with 76 patients completing the study. Milk, beef, pork and egg were the most commonly excluded food types in the true diet group. There was a 3.05 (0.01–6.11) $p < 0.05$ improvement in SIBDQ and 41 (10.4–71.5) in CDAI $p = 0.009$.
Conclusion IgG4-guided exclusion diet, as an adjunct, can improve quality of life and symptoms in patients with CD.

Keywords Crohn's disease · IgG4 · Exclusion diet · Food sensitivity

Introduction

The etiology of Crohn's disease (CD) is considered to be multifactorial and due to an interaction between genetic and environmental factors. The best established environmental risk factors are smoking, appendectomy and use of the oral contraceptive pill [1]. An additional environmental risk factor is diet. Developing countries adopt a Western diet as they become more industrialized and grow wealthier. This transition is thought to be one of the factors that confer an increased risk of developing CD. A similar tendency has recently been reported in a pediatric population with CD: a positive association with a Western diet (meat, fried food, fast foods, snacks and desserts [2].

In patients with active CD, treatment with elemental diet and food exclusion has a similar effect to steroids in inducing remission [3]. Two systematic reviews have been published in recent years examining elemental and polymeric diets in maintaining remission in CD showing favorable results [4, 5]. Elimination diets are also able to maintain remission in patients with CD although their unpalatability has limited their usefulness [6]. Being able to identify which components of the diet which it is most important to avoid is

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therefore an important goal. It has been previously hypothesized that in different patients with CD, different specific protein antigens might be incriminated in the perpetuation of inflammation due to previous sensitization [7]. Trials in subjects with functional gut disorders have found some utility in dietary avoidance guided by IgG4 reactivity [8]. IgG4 is a regulatory subclass of IgG which is produced in response to chronic exposure to antigenic stimulus and hence is an attractive target for the study of food antigen-induced inflammatory responses [9].

Our research group performed the first study using IgG4 reactivity to guide food exclusion in subjects with CD. This was an uncontrolled study, but demonstrated a significant difference in the IgG4 response to certain food antigens between CD and those with irritable bowel syndrome (IBS) and controls [11]. The primary aim of this study was to evaluate the efficacy of an IgG4-guided elimination diet in subjects with CD, on quality of life. This was determined by the Short Inflammatory Bowel Disease Quality of Life Questionnaire (SIBDQ), in a randomized double-blind sham-controlled study. The secondary aim of this study was to assess the effect of the IgG4-guided elimination diet on conventional clinical indices of severity in CD: the Harvey Bradshaw index (HBI), the Crohn's disease activity index (CDAI) and objective measures of inflammation: C-reactive protein (CRP) and fecal calprotectin (FC).

Methods

This study used a double-blinded, randomized, sham-controlled design. All patients with CD reviewed in the outpatient department of three hospitals: one south London teaching hospital and two south London district general hospitals, were considered for inclusion. The diagnosis of CD had been established radiologically or histologically. Clinical disease activity was confirmed by use of both the CDAI and the HBI. For inclusion into the study, each subject required a minimum CDAI score of 80. A cutoff value of 150 is regarded as the point below which a patient is considered to be in remission. However, the reduced value of 80 was used to allow assessment of the effect of the exclusion diet across a broader range of CD patients, since remission based solely on CDAI could not be interpreted as complete. Furthermore, it has been shown that by reducing the cutoff to >80 the diagnostic accuracy can be improved from 41.9 to 67.4 % [11]. Patients with a CDAI >400, a change in medical therapy within 2 months prior to commencing the study or a significant coexisting disease, were excluded from the study. The local ethics committee approved the study, and all patients provided written consent.

Consecutive CD patients reviewed in the outpatient department, who fulfilled the inclusion criteria, were recruited into the study by an assessor. Each patient was assigned a numerical identifier. A dietician who had no clinical contact with the patients in the study held a randomization schedule generated by a random number generation program. The dietician randomized each patient to either a true diet or sham diet group based on the randomization codes and designed either a true or sham diet for each patient depending on which group they had been randomized to.

A true diet sheet excluded the four food types with the highest IgG4 titers, while a sham diet sheet excluded four food types with the lowest titers. The sixteen food types tested for were: milk, peanuts, soya, shrimp, egg, tomato, pork, beef, cod fish, potato, wheat, yeast, cheddar cheese, chicken, lamb and rice. A list of suitable replacement food types was provided on each diet sheet. Thus, cow's milk was replaced with goat's milk and beef was replaced with turkey. Each diet sheet was placed in a sealed envelope labeled with the patient's numerical identifier and sent to the assessor who then forwarded it to the patient. The dietician did not have contact with the patients at any point in the trial. Both the assessor and the patients participating in the trial were blinded to group assignment. Patients were questioned about dietary exclusion compliance at the end of the 4-week diet period.

The primary endpoint of the study was to assess the efficacy of an elimination diet on the quality of life of patients with CD as determined by the SIBDQ. Secondary endpoints assessed include the effect on clinical disease activity as determined by the CDAI and HBI, the effect of a food elimination diet on systemic inflammation measured by CRP and the effect on intestinal inflammation by measurement of FC levels, and the requirement for escalation of medical therapy.

At the screening visit, blood samples were taken for an enzyme-linked immunosorbent assay to detect the presence of IgG4 antibodies specific to the 16 different food antigens and for CRP. A stool sample was collected for measurement of a FC level. Each patient completed a demographic questionnaire, SIBDQ, CDAI and Harvey HBI.

The SIBDQ is a short form of the Inflammatory Bowel Disease Questionnaire (IBDQ), and it has been shown to be a valid and reliable tool that can be used in the routine practical ambulatory care of inflammatory bowel disease (IBD) patients [12]. This questionnaire was developed from the original 32 point IBDQ by the same authors. It utilizes the same seven-point scale, but only ten items-questions have been selected as best predictors of the score. In the seven-point scale used in the response to each item, '7' corresponds to best function and '1' to worst function.

During the 4-week treatment phase, patients were permitted to continue their concomitant medication provided it had been constant for 2 months prior to the start of the study. Each patient was encouraged to adhere to their maintenance drug regimen for the 4-week duration, but those requiring escalation of therapy were withdrawn from the trial and were included in the intention to treat analysis.

IgG4 ELISA

Quantitative measurement of antigen-specific IgG4 antibodies in the patients' serum was performed using the ImmunoCAP-specific IgG4 in vitro test system. The immunoCAP solid phase is the unique part of the test system. It consists of a cellulose derivative containing the antigen of interest. The total binding capacity of the immunoCAP is extremely high, and this ensures binding of all relevant antibodies, regardless of the affinity of the antibody. The cross-reactivity of the enzyme-anti-IgG4 with IgG1, IgG2, IgG3, IgA, IgM and IgE is <0.5 %, which translates as low non-specific binding.

The immunoCAP-specific IgG4 assays were performed using the ImmunoCAP 100E. Blood samples were collected and serum prepared by centrifugation of the blood sample. Serum was stored in a −20 °C freezer. Once an aliquot of serum had been thawed and assayed, it was disposed of to prevent repeated freezing and thawing. Before assaying, the samples were diluted 1:100 with ImmunoCAP-specific IgA/IgG sample diluents. The diluted serum sample was then added to the immunoCap. Food-specific antigen covalently coupled to the ImmunoCAP reacted with the specific IgG4 antibodies in the patient's serum sample. Non-specific IgG4 antibodies were then washed away, and enzyme labeled antibodies against IgG4 were added to form a complex. Following incubation, unbound enzyme-anti-IgG4 was washed away and the bound complex was then incubated with a developing agent. The fluorescence of elute was measured to quantify the specific IgG4 antibodies present in the sample.

Fecal Calprotectin Assay

FC samples were batched and measured at the end. Initial specimens of participants that withdrew from the study were not analyzed. Measurement of the calprotectin level was performed using the Quantum Blue Calprotectin Quantitative Lateral Flow Assay. Stool samples were collected in clean tubes and stored refrigerated at 2 °C. The assay consisted of two individual parts: extraction of the stool samples and the lateral flow assay and reading. In the extraction procedure, the stool sample was placed in the sample chamber in the base cap, which was then attached to the tube. Four milliliters of extraction buffer was then pipetted into the tube and the sample homogenized for 1 min using a Vortex mixer. In order to prepare the sample for measurement, extract was further diluted (1:16) by adding 300 ml of extraction buffer to 20 µl of the extract and homogenized using the Vortex mixer at maximum speed until no large particles were seen. The diluted extract was next centrifuged at 3000 rpm for 5 min. In the second step, the lateral flow assay procedure, 60 µl of diluted and centrifuged stool extract was loaded onto the sample loading port of the test cartridge with a precision pipette and the cartridge placed in the tray of the Quantum Blue Reader®. After 12 min, the test cartridge was read and calculated. Samples with values >300 µg/g were additionally diluted for quantitative determination.

Data Analysis

The primary outcome measure was change in the short IBD quality of life score at week 4 on an intention-to-treat basis. Analysis was also performed per protocol. Changes in the CDAI and HBI were considered secondary outcome measures. The changes in SIBDQ and CDAI scores between the true and sham diets were compared using the unpaired *t* test. Additionally, because of differences in baseline SIBDQ and CDAI values between the groups, a further analysis adjusting for baseline values of SIBDQ in the analysis of SIBDQ and for baseline CDAI in the analysis of CDAI using multiple regressions was performed. Change in HBI, FC and CRP was not normally distributed, and hence, Mann–Whitney *U* test was performed.

Of the 22 patients who did not complete the study, four who withdrew for worsening of symptoms had post-diet disease activity and quality of life scores measured at withdrawal. For the remaining 18 patients, pre-diet scores were imputed as last observation for purpose of intention-to-treat analysis.

Sample Size Calculation

It was calculated that a sample size of 40 patients in each group would be required to detect a difference of ten points on the SIBDQ with 90 % power using a 5 % significance level, and recruitment of a minimum of 100 patients into the trial would allow for an attrition rate of 20.

Results

Patient recruitment and flow through each stage of the study is demonstrated in Fig. 1. Between July 2007 and September 2010, 145 consecutive CD patients were screened for the study, of which 47 (32.4 %) were excluded. Reasons for exclusion from the study include

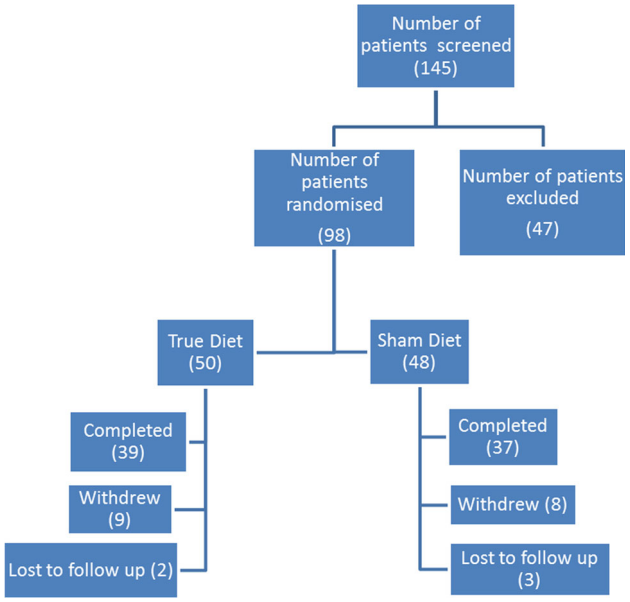

Fig. 1 Diagram to demonstrate the flow of patients through each stage of the study

pregnancy (two patients), clinical remission with a CDAI <80 (43 subjects) and severe clinical disease activity, CDAI >400 (two subjects). The remaining 98 patients were randomized into either a true diet group which received an elimination diet based on their true food sensitivity results or a sham diet group: 50 and 48 patients, respectively. Seventy-six (78 %) patients who completed the protocol were included in the per-protocol analysis; 39 patients from the true diet group and 37 patients from the sham diet group. Of the 22 subjects who did not complete the study, six did not start the study. One of the six was diagnosed with breast cancer and so withdrew, and the other five felt they were unable to commit time to the study and therefore withdrew prior to commencing the diet. Five patients were lost to follow-up; two from the true diet group and three from the sham diet group. The remaining 11 patients started the study, but four withdrew after requiring escalation of medical therapy; three from the true diet arm and one from the sham diet arm of the study. One patient randomized to the true diet arm from the remaining seven had at one time suffered from alcohol dependence and had been abstinent for a period of 12 months but then relapsed and started drinking excessively again 1 week into the study. Two patients who had been randomized into the true diet group withdrew because they found it too difficult to stay on their recommended diets, and the other four patients withdrew because they were no longer able to commit the time to the study: one from the true diet group and three from the sham diet group. In total 17 patients withdrew from the study: nine and eight patients from the true and sham diet groups, respectively. A significant difference had not been demonstrated in the baseline demographics or clinical characteristics of these 17 patients or the five lost to follow-up.

Table 1 shows the baseline demographic details, and the clinical characteristics of all patients randomized into the study ($n = 98$ and those who completed the study ($n = 76$). Baseline characteristics between true and sham were similar with the exception of the CDAI being lower in the true diet group which correlated with higher SIBDQ scores in the true diet group compared to those in the sham diet group. These differences fell short of statistical significance. FC and CRP were only performed in the subjects who started the study. All subjects had an elevated FC at baseline, and the minimum CDAI was 84. Use of immunomodulator therapy was similar in the sham and true diet groups. CRP values and FC samples were not determined on subjects who did not complete the study; therefore, values for intention to treat and per protocol were the same. Correlations between CRP and FC were analyzed by nonparametric Kendall rank correlation test:

CRP and pre-diet SIBDQ $p = 0.03$, CDAI $p = 0.38$, HBI $p = 0.20$
FC and pre-diet SIBDQ $p = 0.19$, CDAI $p = 0.84$, HBI $p = 0.68$.

Table 2 shows the antibody titers to different food types tested in the sham and true groups. It can be seen that titers were highest for four main food groups (beef, pork, egg and milk) and were uniformly low for rice, chicken, tomato and potato, with other food groups demonstrating intermediate reactivity.

Table 3 shows the frequency of different food types excluded in the true and sham diets.

Primary Outcome

Table 4 shows outcomes in patients who completed the study (per protocol) and in all patients (Intention to treat), based on changes in SIBDQ, CDAI, HBI, CRP and FC. There was a significant improvement in SIBDQ, CDAI and HBI by both methods of analysis. We then analyzed a subset of patients with an entry CDAI of >150, and the results are shown in Table 5 of Supplemental material. Similar improvements were found for changes in SIBDQ, CDAI and HBI, but if anything were larger.

A supplementary analysis was performed with a fall in CDAI of >100 as the endpoint. On intention to treat, 6/48 (12.5 %) in the sham versus 16/50 (32 %) in the true experienced a fall in CDAI of this magnitude ($p = 0.028$, Fisher's exact test) and per protocol 6/37 (16 %) in the sham versus 16/39 (41 %) $p = 0.023$ in the true diet group.

There was no significant difference in FC [fall in FC true vs. sham 78.3 μg/l (−33.8 to 190) $p = 0.16$ greater], and

Table 1 Baseline characteristics

	Intention to treat ($n = 98$)		Per protocol ($n = 76$)	
	Sham diet	True diet	Sham diet	True diet
Number of patients	48	50	37 (49 %)	39 (51 %)
Age (median-years) (IQR; range)	36 (21.5; 52)	43.5 (22.0; 59.0)	38 (20.5; 49)	40 (19.75; 52)
Males (%)	20 (42 %)	23 (46 %)	16 (43 %)	16 (41 %)
Smokers (%)	17 (35 %)	17 (34 %)	12 (32 %)	15 (39 %)
Number with family history of IBD (%)	11 (23 %)	10 (20 %)	11 (30 %)	8 (21 %)
Age at diagnosis (median) (IQR; range)			23 (9.5; 45)	25 (16.75; 52)
A1	7 (15 %)	6 (12 %)	5 (13.5)	4 (10 %)
A2	34 (71 %)	34 (68 %)	27 (73 %)	29 (74 %)
A3	7 (15 %)	10 (20 %)	5 (14 %)	6 (15 %)
Disease location				
Ileal	12 (25 %)	14 (28 %)	8 (22 %)	12 (31 %)
Ileocolonic	27 (56 %)	22 (44 %)	20 (54 %)	14 (36 %)
Colonic	9 (19 %)	14 (28 %)	9 (24 %)	13 (33 %)
Number with fistulating disease (%)	4 (8 %)	1 (2 %)	5 (14 %	1 (3 %)
Crohn's related surgery (%)	20 (42 %)	19 (38 %)	18 (49 %)	16 (41 %)
HBI (median) (IQR; range)	7.0 (4.500;16.0)	7.0 (4.0; 15.0)	7.0 (5;14)	7.0 (5; 14)
CDAI (median) (IQR; range)	186 (132;351)	185 (118; 313)	183 (113.75; 272)	181 (123; 308)
SIBDQ (median) (IQR; range)	42 (19.5; 46.0)	50 (16; 41)	42 (16.25; 43)	51 (14.5; 38)
C-reactive protein(mg/dl) (median, range, IQR)	5.8 (0–75, 7.1)	4 (0–23.8, 7.5)	5.8 (0–75, 7.1)	4 (0–23.8, 7.5)
Fecal calprotectin (median, range, IQR)	226 (58–948,357)	388 (58–964, 397)	226 (58–948, 357)	388 (58–964, 397)
Immunomodulator therapy (azathioprine or MTX) (%)	63	80	62	74

Table 2 Mean IgG4 values

Food type	True diet group Mean (median) (IQR; range)	Sham diet group Mean (median) (IQR; range)
Milk α-lactoglobulin	1.59 (0.01) (0.267; 30.0)	2.37 (0.40) (2.587; 22.9)
Milk β-lactoglobulin	3.37 (0.48) (2.605; 30.0)	3.01 (1.430) (3.50; 19.0)
Milk Casein	2.11 (0.48) (1.48; 30.0)	2.38 (0.90) (2.72; 19.890)
Peanuts	0.66 (0.06) (0.305; 7.620)	0.39 (0.110) (0.295; 5.84)
Soya	0.26 (0.20) (0.90; 4.830)	0.28 (0.060) (0.242; 3.59)
Shrimp	0.35 (0.04) (0.092; 10.50)	0.06 (0.030) (0.095; 0.32)
Whole egg	19.6 (26.5) (22.123; 30.0)	20.8 (30.0) (19.25; 29.68)
Tomato	0.59 (0.12) (0.458; 9.210)	0.36 (0.16) (0.352; 2.050)
Pork	0.82 (0.86) (0.552; 2.10)	0.90 (0.94) (0.858; 1.66)
Beef	1.34 (0.67) (0.493; 20.38)	1.81 (0.84) (0.573; 29.68)
Cod fish	0.20 (0.00) (0.057; 4.80)	0.55 (0.010) (0.163; 9.53)
Potato	0.24 (0.040) (0.060; 3.47)	0.10 (0.08) (0.115; 0.50)
Wheat	1.29 (0.31) (0.503; 29.98)	1.38 (0.44) (1.335; 14.86)
Yeast	0.20 (0.10) (0.10; 1.560)	0.15 (0.13) (0.133; 0.47)
Cheddar cheese	2.39 (0.47) (2.105; 30.0)	1.81 (0.98) (2.555; 8.310)
Chicken	0.01 (0.00) (0.00; 0.130)	0.02 (0.00) (0.025; 0.23)
Lamb	0.49 (0.23) (0.275; 8.320)	1.19 (0.32) (0.235; 29.97)
Rice	0.11 (0.010) (0.037; 3.29)	0.12 (0.02) (0.073; 1.98)

Table 3 The frequency of different food types excluded in the true and sham diets

Food type (number of subjects avoiding)	True diet group no. avoiding	Sham diet group no. avoiding
Milk (29)	27	2
Peanuts (14)	4	10
Soya (18)	2	16
Shrimp (24)	2	22
Whole egg (34)	34	0
Tomato (3)	2	1
Pork (22)	22	0
Beef (29)	29	0
Cod fish (26)	6	20
Potato (16)	1	15
Wheat (7)	7	0
Yeast (10)	4	6
Cheddar cheese (16)	14	2
Chicken (33)	0	33
Lamb (3)	3	0
Rice (22)	0	22

for CRP 1.56 mg/l (−5.39 to 8.51). FC and CRP levels were not included in the intention-to-treat analysis. In subset of patients with CDAI >150, there was a significantly greater improvement in FC levels in true versus sham of 142 μg/l (20.0–264) $p = 0.02$, but not for CRP 1.51 (−5.52 to 8.53).

Discussion

Dietary intervention has been used for both acute CD and for maintenance of remission. While some dietary therapies have been shown to be efficacious, the specific mechanisms of action are not clear. Although a number of different diets have been tried to treat CD, only a few are routinely used in the clinical setting: enteral nutrition, low residue diets and exclusion diets. A number of exclusion diets have recently been gaining interest: the specific carbohydrate diet, CD exclusion diets and the IBD anti-inflammatory diet are but a few [13, 14]. However, evidence is lacking in the form of large randomized controlled trials in the CD population. In a recent review article by our group, looking at the mechanisms of dietary influences in CD, we concluded that while there is clear evidence that certain diets are effective in the treatment of CD, the precise components which are important are not well understood, due to a lack of understanding of the underlying pathogenic mechanisms [15].

Our IgG4 sham-controlled diet intervention study demonstrates that after adjustment for baseline SIBDQ scores, there is an improvement in SIBDQ and also an improvement in disease activity scores (CDAI and HBI) with both intention-to-treat and per-protocol analyses. The exclusion of milk, pork, beef and egg was most strongly associated with improvement. The improvements were clinically significant with 41 % of subjects assigned to true diet and only 16 % assigned to sham diet experiencing an improvement in CDAI of >100, an effect similar in magnitude to that seen with more conventional medical therapy.

In all subjects, the markers of systemic inflammation and intestinal inflammation, serum CRP and FC, respectively, did not show a significant reduction between the values in the two groups. However, when data per-protocol patients with a pre-diet CDAI >150 were analyzed, a significant reduction in the FC was observed. This suggests that an IgG4-guided food exclusion diet may have an impact on mucosal inflammation in those with more severe disease.

It was decided to exclude four food types following advice from the dietician that by keeping the number of foods excluded to as few as possible would ensure good compliance. This was supported by experience gained from the pilot study performed by our research group [10]. If all food types the patients reacted to had been looked at then the effect may have been more marked. The study is therefore likely to be a conservative estimate of the true effect of a food elimination diet.

Food antigens may be important in the pathogenesis of CD. We studied a wide range of CD activities, with the lower entry values being below conventional measures of relapse. Indeed, some of these subjects may have had functional symptoms related to their disease. It is not possible to distinguish between the two reasons for symptoms: functional or those related to active CD. Nevertheless, benefits were shown across the range of disease

Table 4 Outcome in all patients (intention to treat) and in patients who completed the study (per protocol)

	Intention to treat (ITT) $n = 98$ (True diet = 50, sham diet = 48)				Per protocol (PP) $n = 76$ (True diet = 39, sham diet = 37)			
	Change in true diet Pre–post-diet score	Change in sham diet Pre–post-diet score	Difference in change true versus sham groups Pre- to post-diet score		Change in true diet Pre- to post-diet score	Change in sham diet Pre- to post-diet score	Difference in change true versus sham groups Pre- to post-diet score	
			Unadjusted (95 % CI)	Adjusted for baseline (95 % CI)			Unadjusted (95 % CI)	Adjusted for baseline (95 % CI)
Change in SIBDQ mean (range, IQR)	−5.78 (−27 to 9, −18 to 0)	−1.07 (−20 to 17, −10.8 to 7.8)	−2.32 (−5.49 to 0.85) $p = 0.15$	−3.05 (−6.11 to 0.01) $p = 0.05$	7.4 (−27 to 9, −18 to 0)	−4.49 (−20 to 17, −18 to 8)	−2.92 (−6.82 to 0.974) $p = 0.14$	−4.728 (−8.10 to −1.36) $p = 0.007$
Change in CDAI mean (range, IQR)	55.7 (−113 to 216)	16.8 (−196 to 196, −98 to 98)	38.9 (8.42–69.5) $p = 0.013$	41.0 (10.4–71.5) $p = 0.009$	−79.4 (−86 to 216, 9.8–141)	−21.21 (−196 to 196, −98 to 98)	58.1 (23.30–93.0) $p = 0.001$	62.8 (28.8–96.8) $p = 0.009$
Change in HBI median (range, IQR)	1 (−1 to 10, 2–5)	0 (−6 to 6, −3 to 3)	1[a] $p = 0.0098$	–	−3 (−1 to 10, 2–7)	−1 (−6 to 6, −3 to 3)	2[a] $p = 0.0093$	–
Change in CRP median (range, IQR)	0 (−12.9 to 17.7, −5.3 to 10.1)	0 (−96.6 to 49.5, −60.1 to 130)	0 $p = 0.2$	–	0 (−12.9 to 17.7, −5.3 to 10.1)	0 (−96 to 50, −60 to 13)	0 $p = 0.19$	–
Change in fecal calprotectin median (range, IQR)	–	–	–	–	35 (−500 to 788, −178 to 466)	−12 (−676 to 716, −328 to 368)	47 $p = 0.13$	–

Difference in changes of SIBDQ and CDAI changes were analyzed using unpaired *t* test. The last column for ITT and PP shows the difference in change adjusted for baseline obtained by multiple regression

[a] Mann–Whitney *U* test was used to measure difference in change of HBI, hence no confidence intervals provided

activities with an emphasis on those with more severe disease.

Importantly to note, no patients in our study had received biologic therapy for their CD. Patients were recruited between 2007 and 2010, with National Institute of Clinical Excellence guidelines on anti-TNF therapy in CD being released toward the end of our recruitment period.

Interpretation

Antibody titers were tested for only 16 common foods, and this panel may not be enough for all patients. Furthermore, an arbitrary cutoff of 0.25 ng/l was used. It is uncertain whether the precise cutoff can be increased or decreased and whether different foods should have different cutoff levels. This remains to be determined. Further studies are needed to determine whether re-challenging patients with the food types initially excluded would result in provocation of their symptoms.

The difference in outcome between the two groups could be explained by the large difference between the two diets rather than by specific identification of food reactions. However, it was the antibody titers themselves which led us to selecting these differences. The four most commonly excluded food types in the true diet group were egg, cheese, milk and beef. However, exclusion of milk was not associated with an improvement in symptoms making lactose intolerance an unlikely explanation for our findings [16, 17].

The wide range of illness severities, which may reflect different origins for symptoms, complicates the interpretation of the study with respect to pathogenesis. However, the effects were observed in all subjects and it is likely that had we restricted the subjects to those with scores conventionally reflecting active disease we would have observed an even stronger effect as demonstrated by the subset analysis of subjects with baseline CDAIs >150. We believe it is likely that similar mucosal inflammatory mechanisms are playing a role even in those subjects with mild symptoms.

We do not believe that the primary abnormality in CD is exposure to food antigens. Diet as a source of luminal antigens is considered to be an important factor in the pathogenesis of CD, but it is yet to be established whether antibodies against dietary antigens have a primary role in CD etiology or are secondary to intestinal inflammation [18–20]. However, food antigens provide a major antigenic challenge to the gut and may lead to immune sensitization resulting in activation of immune cells with resultant mucosal inflammation. Symptoms provoked by certain food may be due either to a local or systemic effect. In the former, vasoactive amines and alkaloids have a direct toxic effect and in the latter there is increased production of pro-inflammatory cytokines such as TNFα, IFNγ and IL4 from peripheral blood mononuclear cells [21]. A further mechanism by which an exclusion diet may affect disease activity is through manipulation of gut micro flora with enhancement of beneficial bacteria in the gut [22, 23].

It has been previously suggested that the presence of IgG4 antibodies against food antigens ought to be interpreted as a result of antigen exposure, rather than disease [24]. Antibody levels against food components are not solely determined by exposure through a breached epithelium. This is suggested by the high prevalence of the anti-yeast antibodies (anti-saccharomyces cerevisiae antibody) in CD and their low prevalence in ulcerative colitis. The mean IgG4 response against rice was one of the lowest, and this was true even for those patients of Asian origin for whom rice is part of the staple diet.

The production of large quantities of IgE are not required for food intolerance or food hypersensitivity since in humans IgG4 is also able to bind mast cells, thereby inducing mast cell degranulation with release of stored mediators which not only result in mucous hypersecretion and increased vascular permeability but also recruit other inflammatory cells such as eosinophils and neutrophils. Increased mast cell numbers and elevated levels of mast cell mediators in both the intestinal lumen and tissue have been demonstrated in CD [25, 26]. Further understanding of the mechanisms underlying food intolerance may lead to more successful treatment of CD in the future.

Limitations of the Study

This was a relatively small study and only achieved its primary endpoint after adjustments were made for the imbalances in baseline values in the SIBDQ. However, the consistency with the other two clinical scores the CDAI and HBI in finding benefit suggests that this is a true effect.

Although the primary aim was to assess quality of life, the inclusion criteria were based on CDAI score rather than SIBDQ score. Though this may be confusing, this was done as more research studies have used CDAI scoring as a valid means of participant selection.

The period of follow-up was only 4 weeks, and therefore, it is difficult to say whether benefit ascertained from the IgG4-guided exclusion diet was short lived. Further work with follow-up at longer periods is required to determine the long-term efficacy of such a diet.

It is difficult to be certain of patient compliance to the exclusion diets, as no objective measure of compliance was ascertained. However, participants were motivated to trial the diets as they felt that their illness was affected by diet. Importantly, non-compliance in either group would only lessen the chances of observing any differences. In other ongoing work in IgG4-based exclusion diets by our group, participants are now asked to keep a food diary as a means of aiding and establishing compliance, and avoiding this limitation.

The primary endpoint in this study was clinical, but it would be desirable to replicate these findings for biological markers of disease activity. The study was not powered to look at these, but among subjects with a baseline CDAI over >150 there was a significant improvement in FC and trend toward a change in CRP. However, the precise relation between these biological markers and the symptoms which we wish to treat is uncertain. Hence, the validity of the study for clinical practice is undiminished.

Practical Implications of the Study

Non-compliance can be a major problem, and it requires highly motivated patients for the treatment to be successful. In clinical practice, a targeted approach such as adopted in this study has the advantage of excluding only those foods that are associated with increased antibody titers. An individualized diet obviates the need for exclusion of a large number of foods, and this would hopefully increase the likelihood of compliance. A further benefit of a targeted approach is prevention of malnutrition, which may occur as a consequence of unsupervized dietary restriction.

It may also be that it is only necessary to exclude the main culprit antigens identified in this study, such as red meat and eggs. This could usefully be the subject of future investigation.

Conclusion

Dietary modification through identification of offending foods by IgG4 ELISA results in clinical benefit, which is most likely at least in those with the most active disease to be as a consequence of reducing both systemic and local bowel wall inflammation. An IgG4-guided exclusion diet may provide a further method of treatment for well-motivated and determined patients with Cd, thereby avoiding complications of drugs and unnecessary surgery as well as the difficulties in following elemental diets which cannot be maintained long term. These findings require verification in a large, randomized, controlled trial.

Compliance with ethical standards

Conflict of interest The authors declare that there are no competing interests.

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