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ORIGINAL ARTICLE

Glutamine and Whey Protein Improve Intestinal Permeability and Morphology in Patients with Crohn's Disease: A Randomized Controlled Trial

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Abstract
Background Increased intestinal permeability (IP) has been implicated in the etiopathogenesis, disease activity and relapse of Crohn's disease (CD). Glutamine, the major fuel for the enterocytes, may improve IP.
Aim We evaluated the effect of oral glutamine on IP and intestinal morphology in patients with CD.
Methods In a randomized controlled trial, consecutive patients with CD in remission phase with an abnormal IP were randomized to a glutamine group (GG) or active control group (ACG) and were given oral glutamine or whey protein, respectively, as 0.5 g/kg ideal body weight/day for 2 months. IP was assessed by the lactulose mannitol excretion ratio (LMR) in urine, and morphometry was performed by computerized image analysis system.
Results Patients (age 34.5 ± 10.5 years; 20 males) were assigned to the GG ($n = 15$) or ACG ($n = 15$). Fourteen patients in each group completed the trial. The LMR [median (range)] in GG and ACG at 2 months was 0.029 (0.006–0.090) and 0.033 (0.009–0.077), respectively, with $P = 0.6133$. IP normalized in 8 (57.1%) patients in each group ($P = 1.000$). The villous crypt ratio (VCR) [mean (SD)] in GG and ACG at 2 months was 2.68 (1.02) and 2.49 (0.67), respectively, ($P = 0.347$). At the end of 2 months LMR improved significantly in GG from 0.071 (0.041–0.254) to 0.029 (0.006–0.090) ($P = 0.0012$) and in ACG from 0.067 (0.040–0.136) to 0.033 (0.009–0.077) ($P = 0.0063$). VCR improved in the GG from 2.33 (0.77) to 2.68 (1.02) ($P = 0.001$), and in ACG from 2.26 (0.57) to 2.49 (0.67) ($P = 0.009$).
Conclusions Intestinal permeability and morphology improved significantly in both glutamine and ACG.

Keywords Crohn's disease · Glutamine · Intestinal morphology · Intestinal permeability · Lactulose mannitol ratio · Villous crypt ratio

Introduction

Crohn's disease (CD) is a chronic inflammatory condition, which may involve any part of the digestive tract. Intestinal permeability (IP) is a measure of the barrier function of the gut which relates to the vast paracellular space surrounding the brush border surface of the enterocytes and the tight junctions (TJs). Increased IP is a common feature in about

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30–65% of patients with CD [1–4]. IP has been implicated in the etiopathogenesis of CD, as the alterations in IP are seen in 25% of healthy first degree relatives of CD, who may develop CD eventually [5, 6]. Abnormal IP has been associated with early relapse with a sevenfold increase in the relapse rates in patients with increased IP compared to normal IP [7]. Abnormal IP also precedes clinical flare-ups [8]; hence, it is suggested as a marker of impending relapse. Besides this, the magnitude of alteration in IP also correlates with the activity of the disease [9], where improvement in the disease activity corresponds with improvement in IP after therapy [10]. Thus maintenance of normal IP may have a therapeutic benefit on the natural course of the disease.

Glutamine, the most abundant amino acid in the body, is the principal fuel and a nitrogen source for the enterocytes. The human intestine extracts approximately 13% of the circulating glutamine [11], which prevents intestinal hyperpermeability, bacterial translocation and maintains intestinal mucosal integrity in a variety of diseases [12–16]. The benefits of glutamine have been described in a number of experimental and human studies, using either enteral [17–20] or parenteral [21–23] glutamine. Glutamine exerts specific effects on the tight junction (TJ) proteins and influences the intracellular mediators thereby enhancing the TJ resistance [24]. It also prevents the acetaldehyde induced injury to the TJ proteins [25]. It reorganizes and redistributes these proteins and thereby prevents the paracellular hyperpermeability and endotoxin permeation. Glutamine also stimulates the intestinal mucosal hyperplasia and cell migration concomitantly inhibiting enterocyte apoptosis [26].

Though a few studies on experimental models of colitis have shown that glutamine improves mucosal permeability, decreases endotoxin levels, and reduces bacterial translocation, it nevertheless lacks corroboration from human studies in inflammatory bowel disease. So far only two studies [27, 28] have shown the beneficial effect of glutamine in maintaining IP in CD. Of these, one is an uncontrolled study published only as an abstract [27], which showed improvement in IP after oral glutamine supplementation and the other showed maintenance of normal IP with glutamine supplemented TPN compared to standard TPN [28]. Thus, despite the potential benefits of glutamine, there is indeed a lack of convincing clinical evidence for glutamine supplementation to improve IP in CD. Undoubtedly, IP is an important aspect in CD, therefore normalization of IP remains a worthwhile therapeutic objective, and glutamine has a promising role in preserving and restoring normal IP. Consequently this RCT was planned with the objective to see the effect of oral glutamine supplementation on the IP, and morphology in patients with CD.

Materials and Methods

Study Design

This was a randomized, controlled open-label trial.

Locale

This study was performed in a tertiary care medical center at New Delhi, India.

Patients

All consecutive patients with CD attending the Gastroenterology OPD and the IBD clinic were enrolled in the study from November 2005 to November 2008 as per the inclusion criteria.

Diagnosis of CD

The diagnosis of CD was based on the standard diagnostic criteria, which included clinical evaluation in combination with endoscopic, histological and radiological features.

Inclusion Criteria

All consecutive patients with CD in the remission phase with an abnormal IP were randomized into the trial. Patients were in the remission phase if their Crohn's disease activity index (CDAI) was <150. IP was considered abnormal if the ratio of lactulose and mannitol (LMR) in urine was >0.037 (details given further).

Exclusion Criteria

Patients with the following conditions were excluded: (a) having an active disease, i.e. CDAI score >150, (b) having a normal IP, (c) taking a high protein nutritional supplement or those who have taken protein supplement in the preceding 3 months, (d) associated systemic diseases like chronic liver disease (AST/ALT more than twice the normal for two consecutive times and/or serum bilirubin >2.0 mg%) and kidney disease (blood urea >60 mg% and/or serum creatinine >1.8 mg%), diabetes mellitus (fasting plasma glucose >126 mg/dl and/or 2 h post prandial glucose >200 mg/dl after a 75 g glucose load) and malignancy, (e) pregnancy or lactation and (f) age <15 and >60 years.

Controls

Fifty healthy controls (HC) were taken to determine the upper limit of normality or cut-off of LMR and 20 HC for

assessment of plasma glutamine. These HC were free from any gastrointestinal disorder or other systemic disease. None had a history of intake of NSAID, alcohol or protein supplements and smoking.

Study Plan

Once the diagnosis was confirmed, all the patients underwent the IP test. Those who fulfilled the inclusion criteria for intervention, were randomized for the trial and were followed-up at regular intervals in the Gastroenterology OPD.

Management of CD

All the patients were treated with standard drug regimen, including mesalamine, corticosteroids and azathioprine along with hematinics, multivitamins and calcium as per indications. No change was made in the usual drug therapy during the period of intervention.

Randomization (Sequence Generation and Allocation Concealment)

An external third party randomization procedure was used. A randomization list was generated by a Statistician using STATA 9.0 software (College Station, TX, USA). Block randomization was used to allocate the patients to the glutamine and active control group (ACG). Allocation of the patients to receive the treatments was done by sequentially numbered opaque sealed envelope (SNOSE) method. The envelopes were prepared by a person not associated with the conduct of the study.

Intervention

The total energy requirement was supplied in the form of protein, carbohydrates and fats amounting to 20, 55, and 25%, respectively.

Glutamine Group

One third (0.5 g/kg ideal body weight/day) of the protein requirement of these patients was provided in the form of a water soluble commercial preparation (Glutammune, Claris lifescience Ltd, India) of pure L-glutamine, containing 100% glutamine. Each packet of 10 g glutamine powder (costing Rs. 125) provided 10 g protein and 40 kcal.

Active Control Group

One third (0.5 g/kg ideal body weight/day) of the protein requirement of these patients was provided in the form of a water soluble preparation of whey protein concentrate (Mahaan Proteins Ltd, India) containing 70% protein, 14% carbohydrates, 5% fat, 6% minerals and 5% moisture. In-house packaging of whey protein powder was done into packets of 10 g (costing Rs. 15) each providing approximately 7 g protein and 38 kcal.

The remaining 1 g/kg ideal body weight/day of protein was met by a normal home-made diet in both the groups. Intervention was given for a period of 2 months.

Nutritional Assessment

The dietary intake was assessed by the 7-day dietary diary method [29]. Macronutrients were calculated with the help of software—computerized nutrient evaluation program (CNIEP)—which was based on the Indian food composition tables by the Indian Council of Medical Research [30]. The difference of the daily requirements and actual intake of macronutrients were met by a diet planned as per individual food habits and income status. The diet charts also specified the amount, timing and the method of consumption of the protein supplements. Height, weight and tricep skin fold (TSF) were measured and BMI was calculated.

Assessment of Compliance

Adherence to the interventions along with the dietary regimen was checked every 15 days, either on phone or by personal visits. All the patients were asked to keep a daily diet and supplement diary until the completion of intervention. Individual diet charts and menus provided to the patients ensured proper understanding. Compliance was assessed by asking target questions and crosschecking with the nearest relative staying with them, return of empty sachets of glutamine and whey protein and daily record of supplements.

Safety Evaluation

All the patients were monitored for adverse reactions due to the intervention. The safety and tolerability of the intervention was judged by regular physical examination, blood biochemistry and monitoring the symptoms as recorded by the patient in the diary.

Follow-up Assessment

The clinical details, liver and kidney function tests, IP test, morphometry of the duodenal biopsies, assessment of plasma glutamine and nutritional status were repeated after intervention, at 2 months.

Primary Outcome Parameters

Improvement in IP was used as an outcome parameter as assessed by LMR.

Secondary Outcome Parameters

1. Change in the intestinal morphology.
2. Change in plasma glutamine levels.

Assessment of Intestinal Permeability

Test Procedure

After an overnight fast, patients collected a pre-test mid stream urine sample, evacuated the bladder, and drank the test solution containing 5 g of lactulose, 2 g of mannitol and 5 g of D-xylose in 100 ml water. No food or drink was allowed until the completion of the test. Only water was permitted 1 h after the ingestion of the test solution. All the urine passed in the subsequent 5 h was collected in a plastic can containing 20% chlorohexidine. The total volume of urine was noted and 15 ml aliquots were stored at −20°C.

Estimation of Mannitol

Mannitol assay in urine was done by the method described by Corcoran and Page [31], which was based on the measurement of formaldehyde produced after the oxidation of mannitol by periodic acid.

Estimation of Lactulose

The lactulose assay was based on the method of Behrens et al. [32], in which lactulose is hydrolysed by β galactosidase to fructose and galactose. Subsequently, by a series of enzymatic reactions, fructose is converted to gluconate 6 phosphate and NADPH. The amount of NADPH is directly proportional to the amount of lactulose in urine which is measured by change in absorbance at 340 nm.

Analysis of Urine Samples

The baseline samples were analyzed consecutively in an un-blinded manner. The post intervention samples were analyzed in a blinded fashion. All the samples were given a unique four-digit alphanumeric code. A list of these codes was maintained by a single operator not associated with the trial. At the end of the trial the labels were uncovered and codes were identified with the patient names.

Estimation of Cut-Off for LMR

The results of the LMR in 50 HC were used to fix the upper limit of IP at one tailed 90% tolerance interval. Since the distribution of LMR in controls was non-normal, a square root-transformation was carried out and subsequently the cut-off value was established as mean +1.28 SD of the square root of LMR. The obtained cut-off of LMR in HC is 0.037 which corresponds to the 90th percentile. LMR $\leq$ 0.037 was normal and >0.037 was considered as abnormal IP.

Morphometry

Procurement of Duodenal Biopsies

Four biopsies were obtained from beyond the distal end of the second part to the third part of the duodenum by video endoscope (Olympus Opticals Company, Japan) and a disposable biopsy forceps (Wilson-Cook, NC, USA). Ten sections (3 µ each) were stained with hematoxylin and eosin and slides were prepared for histological examination.

All biopsies were processed by a single technician. Slides were evaluated by a single senior pathologist and morphometry was performed by a single observer in a blinded manner.

Quantitative Assessment of Biopsies

A computerized image analysis system comprising a research light microscope (BX50, Olympus Corporation, Japan), a digital camera (CoolSnapProcf Color, Media Cybernetics Corporation, USA), a personal computer with Pentium IV Intel processor and image analysis software (Image ProPlus Ver 6.0.0 [2006]) was used for quantitative assessment of parameters.

Capturing Images

For each biopsy one complete set of images, comprised of well oriented villi and the corresponding crypts under a 40× magnification, enterocytes under 400× and muscle under 100× magnifications, was captured under uniform illumination and condenser settings.

Inclusion and Exclusion Criteria

Areas which had at least four consecutively ideally oriented villi, i.e. vertically oriented, with a corresponding crypt visible in continuity without any artifacts of sectioning were selected. Villi which were sectioned tangentially were excluded. If two villi were partly or fully fused,

they were treated as one. Ten paired biopsies in each group were adequate for morphometry.

Image Measurement

Villus Height

Villus height (VH) was measured from the tip of the villus-crypt junction to the lumen (*A* in Fig. 1). The villus-crypt junction was determined by the presence of the "shoulder" and by narrowing of the villous lamina propria subsequent to that.

Crypt Depth

The crypts were measured one by one alongside the corresponding villi as the distance between the open "mouth" of the crypt through the center of the crypt to the basement membrane zone at the base of the crypt (*D* in Fig. 1).

Villous Width

Villous width (VW) was measured as the broadest part of the villus along a line perpendicular to VH above the villous shoulder in each unit of the villous compartment (*B* in Fig. 1).

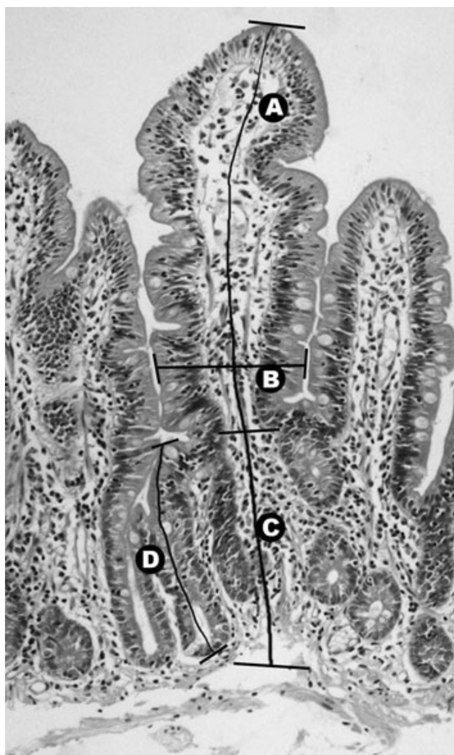


Fig. 1 Image measurement (H&E ×40). *A* villus height, *B* villus width, *C* total mucosal thickness, *D* crypt depth

Total Mucosal Thickness

Total mucosal thickness (TMT) was measured from the tip of the well oriented villus running along the longitudinal axis to the inner aspect of the muscularis mucosa (*C* in Fig. 1).

Muscle Thickness

A minimum of four measurements were obtained along the stretch of the muscularis mucosa, preferably where the overlying crypts were vertically oriented and the muscle bundles were not tangentially cut. Care was taken to exclude areas of the biopsies where the muscularis showed any stretching.

Enterocyte Height

Ten adequately oriented enterocytes were measured predominantly in the middle third of the villus from the villus-crypt junction. Selected cells had oval, basal nuclei with minimal or no sectioning artifacts. Height was measured from the basement membrane up to the glycocalyx (excluding the glycocalyx).

Intra Epithelial Lymphocytes

Intra epithelial lymphocytes (IELs) are seen in the epithelium as darkly stained nuclei which are characteristically basally located with a clear cytoplasmic halo around them (Fig. 2). The number of lymphocytes lying within the epithelium of villi for a total of 500 enterocytes was counted. The lowest quarter of each villus was excluded from the count. Results were expressed as IELs per 100 enterocytes.

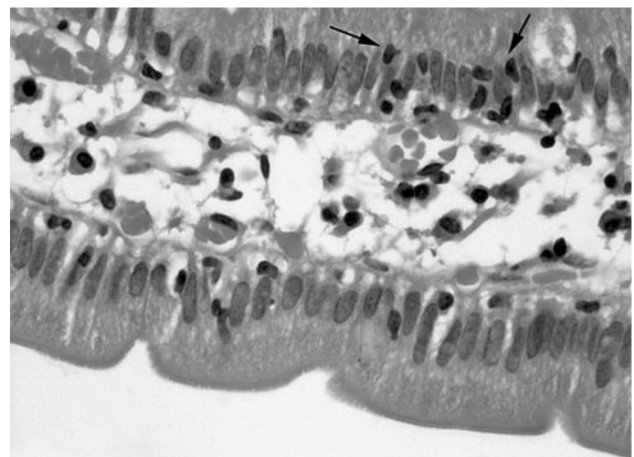


Fig. 2 Intraepithelial lymphocytes (IELs) (H&E ×100)

Plasma Glutamine

Plasma glutamine was estimated in 20 patients (10 in each group) by the HPLC method by Teerlink et al. [33].

Statistical Analysis

Sample Size Determination

The sample size calculation was based on the previous study by Zoli et al. [27], where the LMR was reduced from a median value of 3.7–0.8 after oral glutamine supplementation. Considering 78% reduction in the glutamine group (GG), based on this study, with a high SD of 55% and assuming 20% reduction in ACG, with 5% level of significance and 80% power, the estimated sample size was 15 in each group.

Analysis

Statistical analysis was carried out using STATA 9.0 (College Station, TX, USA). Data were presented as number (%) or mean ± SD/median (range) as appropriate. Categorical and continuous baseline characteristics between the groups were compared using Chi square test/Fisher's exact test or Wilcoxon rank sum test or Student's *t* test as appropriate. The analysis was per protocol since two patients were not included in the analysis. The effect of glutamine as compared to active control on IP (LMR) was compared using Wilcoxon rank sum test since the data were non-normal. The change in IP (LMR) from baseline was compared in each group using Wilcoxon signed rank test. The morphometric parameters were compared between the groups using generalized estimating equation (GEE) since the observations were correlated. A P value less than 0.05 was considered statistically significant.

Ethical Considerations

The protocol of the study was approved by the institutional Ethics Committee (Reference number-A-14/4.8.2004). Written informed consent was taken from all the patients before performing the IP test and again from those participating in the trial after explaining the nature and purpose of the trial. Patients were also provided the patient information sheet explaining the benefits and risks of the trial. This study has been registered at the clinicaltrials.gov; the identifier for our study is NCT00838149.

Results

One hundred sixty-two patients with CD were screened, of these only 40 patients fulfilled the inclusion criteria. Eight of these 40 refused to participate in the trial, while two patients were unable to come for follow-up, and hence were not included. Thus, 30 patients were randomized, 15 in the GG and 15 in the ACG. Twenty-eight of these 30 completed the intervention for a period of 2 months. One patient in the GG was withdrawn due to increased stool frequency and one patient in the ACG was lost to follow-up. Finally, 28 patients, 14 in each group, who completed the trial, were included in the analysis as shown in the CONSORT flow chart (Fig. 3).

Baseline Demographic, Clinical, and Biochemical Parameters

The mean age of the patients, gender distribution, location, behavior, duration and age at onset of the disease, CDAI score, presence of extra-intestinal manifestations, nutritional status and routine biochemical investigations were comparable between the groups at baseline (Table 1). Four patients out of those randomized in the trial had a history of surgery; however, they did not have any symptoms suggestive of malabsorption.

Effects of Intervention

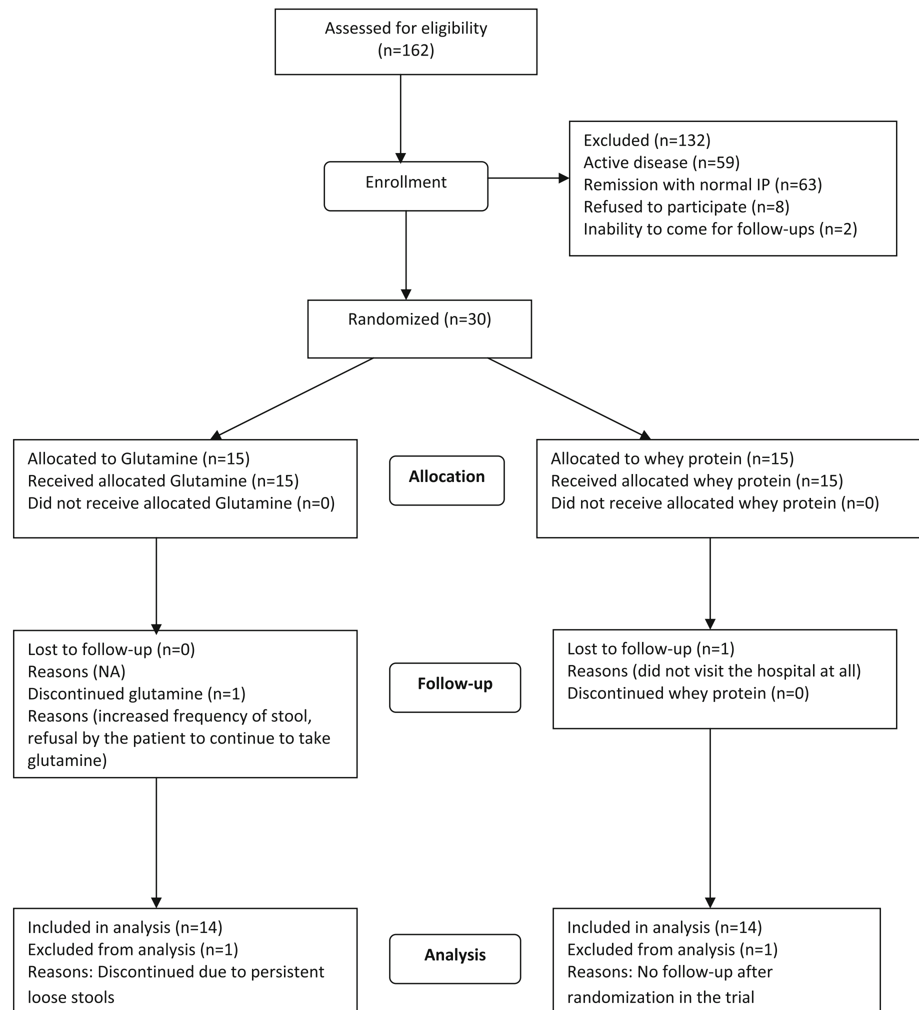
Intestinal Permeability (IP)

The %L, %M excretion and the LMR between GG and ACG were not significantly different at baseline and after 2 months. The LMR decreased from a median value of 0.071 (0.041–0.254) to 0.029 (0.006–0.090) in the GG, whereas it decreased from a median value of 0.067 (0.040–0.136) to 0.033 (0.009–0.077) in the ACG. After intervention, the IP normalized in 8 (57.1%) patients, each in GG and ACG ($P = 1.00$) (Table 2). Individual changes in LMR from baseline to 2 months in both groups are shown in Fig. 4.

Intestinal Morphology and IELs

The VH, VW, crypt depth (CrD), TMT, muscle thickness (MT) and enterocyte height (EH) were not significantly different in both the groups at 2 months after intervention. However, in both the GG and ACG there was a significant increase in the villous crypt ratio (VCR) after intervention [GG: baseline vs. 2 months = 2.33 (0.77) vs. 2.68 (1.02), $P = 0.0001$; and ACG: baseline vs. 2 months = 2.26 (0.57) vs. 2.49 (0.67), $P = 0.009$]. At baseline both the groups had a similar distribution of IELs. At the end of 2 months the IELs decreased in both the groups, yet it did not reach a level of statistical significance (Table 3).

Fig. 3 CONSORT flowchart



Plasma Glutamine

There was no significant difference in the plasma glutamine levels of the patients and the HC (patients vs. HC = 776.2 ± 162 vs. 735.6 ± 104; $P = 0.354$). At baseline and at the end of 2 months the level of plasma glutamine was comparable between the GG and ACG. The change in the plasma glutamine levels of the patients in both the groups over a period of 2 months was not significant [GG: baseline vs. 2 months = 767.0 ± 80.2 vs. 763.0 ± 170, $P = 0.857$; and ACG: baseline vs. 2 months = 781 ± 223.0 vs. 769.1 ± 143.2, $P = 0.390$].

Activity of the Disease

At baseline all the patients were in the remission phase of the disease, having a comparable CDAI score. Over a period of 2 months, two patients in each group went into the active phase of the disease ($P = 1.000$). The median CDAI score at the end of 2 months was also comparable between the groups [GG vs. ACG = 135.0 (5.5–234.0) vs. 101.0 (15.2–247.0), $P = 0.7828$].

Adverse Reactions

A total of nine patients (7 [50.0%] in the GG and 2 [14.3%] in the ACG) had an increase in the stool frequency, from 1–2 stools per day to 3–5 stools per day.

Nutrient Intake

The protein and calorie intake increased from 65.2 ± 27.5 to 93.2 ± 25.6 g/day ($P = 0.0004$) and 2,042 ± 738 to 2,203 ± 648 kcal ($P = 0.2994$), respectively, in the GG, and from 60.4 ± 23.6 to 85.4 ± 20.0 g/day ($P = 0.0001$) and 1,833 ± 533.5 to 2,154 ± 455 kcal/day, ($P = 0.0107$), respectively, in the ACG. However there was no difference in the protein and calorie intake between both the groups at 2 months.

Table 1 Baseline demographic, clinical, disease characteristics, and routine biochemical investigations of the patients in the glutamine and active control groups

Variables	Glutamine (n = 15)	Active control (n = 15)	P value
Age (years)	35.1 ± 10.8	33.9 ± 10.4	0.7597
Gender (M:F)	10 (66.7):5 (33.3)	10 (66.7):5 (33.3)	1.000
Location of the disease			
Terminal ileum (L1)	1 (6.7)	2 (13.3)	0.500
Colon (L2)	1 (6.7)	5 (33.3)	
Ileo-colon (L3)	6 (40.0)	4 (26.7)	
Terminal ileum + UGI (L1, L4)	2 (13.3)	1 (6.7)	
Colon + UGI (L2, L4)	1 (6.7)	0 (0.0)	
Ileo-colon + UGI (L3, L4)	4 (26.6)	3 (20.0)	
Behavior of the disease			
Non-stricturing nonpenetrating (B1)	6 (40.0)	6 (40.0)	0.847
Stricturing (B2)	9 (60.0)	7 (46.6)	
Penetrating (B3)	0 (0.0)	1 (6.7)	
Nonstricturing nonpenetrating + perianal (B1,P)	0 (0.0)	1 (6.7)	
Stricturing + perianal (B2, P)	0 (0.0)	0 (0.0)	
Penetrating + perianal (B3, P)	0 (0.0)	0 (0.0)	
Extraintestinal manifestations	7 (46.7)	3 (20.0)	0.245
Duration of the disease			
Duration (months)	60 (13–180)	72 (6–144)	0.8843
≤36 months	5 (33.3)	6 (40.0)	1.000
>36 months	10 (66.7)	9 (60.0)	
Age of onset of disease			
Age of onset (years)	26 (9–48)	27 (15–50)	0.9834
(A1) <16 years	1 (6.7)	1 (6.7)	1.000
(A2) 17–40 years	11 (73.3)	12 (80.0)	
(A3) >40 years	3 (20.0)	2 (13.3)	
CDAI score	76.9 (13.4–145.0)	93.3 (24.0–141.0)	0.8682
Diet and nutritional status			
Energy (kcal/day)	2,042 ± 738	1,833 ± 533	0.2516
Protein (g/day)	65.2 ± 27.5	60.4 ± 23.6	0.4634
Weight (kg)	56 (39.6–72.0)	52.4 (30.0–72.0)	0.3837
BMI (kg/m^2)	21 (16.3–24.8)	19.8 (14.1–24.3)	0.5896
TSF (mm)	11.5 (6.2–26.5)	8.6 (5.2–27.8)	0.3723
Serum biochemistry			
Hemoglobin (g/dl)	11.1 ± 2.3	10.9 ± 1.9	0.8411
Platelets ($\times 10^3$)	244 (151–730)	240 (173–373)	0.5067
TLC (cumm)	7,800 (4,200–19,400)	6,400 (3,300–16,400)	0.3092
ESR (mm/1st hour)	40 (1–52)	30 (2–55)	0.5061
PCV	35 (19–46)	34 (23–47)	0.9501
S urea (mg/dl)	18 (14–60)	18 (14–32)	0.8998
S creatinine (mg/dl)	0.920 ± 0.185	0.957 ± 0.169	0.9257
Sodium (mEq/l)	139.8 ± 4.9	141.0 ± 4.5	0.4156
Potassium(mEq/l)	4.4 ± 0.55	4.3 ± 0.39	0.7623
Uric acid (mg/dl)	5.1 ± 1.68	5.1 ± 1.02	0.8358
Calcium (mg/dl)	9.0 ± 0.71	9.4 ± 0.72	0.1549
Phosphate (mg/dl)	3.6 ± 0.66	3.9 ± 0.85	0.3915
S bilirubin (mg/dl)	0.8 ± 0.36	0.7 ± 0.16	0.2963
T protein (g/dl)	7.5 ± 0.76	7.5 ± 0.60	0.9361

Table 1 continued

Variables	Glutamine ($n = 15$)	Active control ($n = 15$)	P value
Albumin (g/dl)	4.2 ± 0.59	4.3 ± 0.52	0.7425
Globulin (g/dl)	3.2 ± 0.64	3.2 ± 0.65	0.7567
SGOT (IU)	33 (19–45)	39 (23–117)	0.2894
SGPT (IU)	22 (12–58)	29 (12–56)	0.4668
Alkaline phosphatase (IU)	200 (81–340)	192 (71–641)	0.9504

Data are expressed as mean ± standard deviation (SD) or median (range) and number (percentage)

Table 2 Comparison of intestinal permeability (IP) between glutamine (GG) and active control groups (ACG) after intervention

Variable	n	Glutamine group	Active control group	P value
Lactulose excretion (%)				
Baseline	15	0.725 (0.102–1.80)	0.707 (0.269–3.19)	0.8357
2 months	14	0.536 (0.091–1.15)	0.501 (0.199–1.95)	0.7477
P value		0.0186*	0.0413*	
Mannitol excretion (%)				
Baseline	15	10.4 (1.05–22.4)	9.9 (2.5–23.8)	1.000
2 months	14	17.7 (3.0–27.6)	17.5 (6.4–44.2)	0.9450
P value		0.0026*	0.0186*	
Lactulose mannitol ratio				
Baseline	15	0.071 (0.041–0.254)	0.067 (0.040–0.136)	0.9835
2 months	14	0.029 (0.006–0.090)	0.033 (0.009–0.077)	0.6133
P value		0.0012*	0.0063*	
IP at 2 months				
Normal		8 (57.1)	8 (57.1)	1.000
Abnormal		6 (42.9)	6 (42.9)	
Difference in proportion of abnormal IP (95% CI)		0.0 (−36.7, 36.7)		

Data expressed as median (range) or number (percentage), and 95% CI; *CI* confidence interval

* Significant at $P < 0.05$

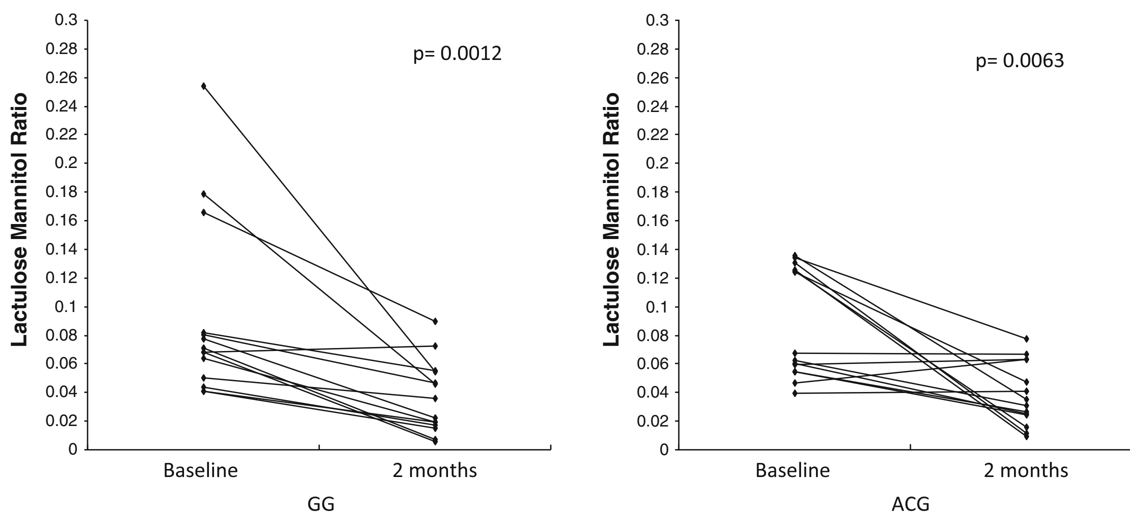


Fig. 4 Individual changes in LMR from baseline to 2 months in glutamine (GG) and active control groups (ACG)

Discussion

In view of the far reaching role of IP in CD it is plausible that maintaining normal IP should have a major therapeutic advantage. Glutamine serves as the principle fuel and nitrogen source for the enterocytes. In a number of experimental studies [17, 18, 20, 23] both enteral and parenteral glutamine have been reported to improve IP,

Table 3 Comparison of intestinal morphology and intra epithelial lymphocytes (IELs) between glutamine and active control groups (ACG) at 2 months

Variable	Mean (SD)		Difference (95% CI)	*P* value
	Glutamine group ($n = 10$)	Active control group ($n = 10$)		
Villus height (μm)				
Baseline	466.0 (105.5)	437.4 (98.4)		0.196
2 months	502.1 (143.2)	459.2 (101.5)	42.9 (−10.5,96.4)	0.116
P value	0.143	0.299		
Villus width (μm)				
Baseline	142.0 (39.0)	148.6 (41.1)		0.460
2 months	154.8 (43.4)	150.8 (32.6)	3.95 (−11.4,19.3)	0.615
P value	0.090	0.723		
Crypt depth (μm)				
Baseline	201.5 (45.7)	192.7 (37.2)		0.487
2 months	184.5 (33.0)	191.8 (40.1)	7.2 (−11.9, 26.4)	0.460
P value	0.129	0.909		
Mucosal thickness (μm)				
Baseline	703 (135.1)	642 (128.0)		
2 months	708 (162.1)	680 (124.7)	28 (−40.6,96.8)	0.056
P value	0.864	0.165		0.423
Muscle thickness (μm)				
Baseline	57.4 (16.2)	53.4 (15.4)		0.518
2 months	50.1 (13.4)	51.5 (17.2)	1.38 (−10.4,13.2)	0.817
P value	0.141	0.742		
Villus crypt ratio				
Baseline	2.33 (0.77)	2.26 (0.57)		0.688
2 months	2.68 (1.02)	2.49 (0.67)	0.187 (−0.203,0.579)	0.347
P value	0.0001*	0.009*		
Enterocyte height (μm)				
Baseline	32.8 (6.9)	30.3 (5.2)		0.212
2 months	33.0 (4.02)	31.6 (4.4)	1.36 (−2.10,4.83)	0.442
P value	0.893	0.492		
IELs				
Baseline ($n = 15$)	38.1 (13.8–68.8)	38.0 (17.6–63.8)		0.2213
2 months ($n = 14$)	29.6 (18.0–59.0)	32.1 (25–44.8)		0.1410
P value	0.2024	0.0884		

Data are expressed as mean (SD) and median (range)

* Significant $P < 0.05$

prevent bacterial translocation and reduce the endotoxin levels. Likewise, the positive effect of enteral/parenteral glutamine on the gut barrier function has been observed in a multiplicity of clinical conditions [12, 14–16, 34]; however, the speculative benefits of glutamine on IP and morphology, described in the experimental models of colitis [35–37], lack corroboration in case of IBD. So far, out of the five studies [27, 28, 38, 39, 40] examining the effect of enteral or parenteral glutamine on IP in CD, only two studies [27, 28] have shown a beneficial role of glutamine. This RCT was conducted to see the effect of oral glutamine on IP and intestinal morphology in CD. We observed a significant improvement in IP (LMR) and intestinal morphology (VCR) in the GG; however, a similar effect was also seen in ACG.

In previous studies a maximum dose of 21 g of glutamine, given for a period of 4 weeks did not show any beneficial effect [38]; hence we selected a relatively higher dose as 0.5 g/kg IBW/day. In the literature no adverse effects have been demonstrated at doses up to 50–60 g/day given over a number of weeks [41, 42]. In order to balance the ACG in terms of the net protein intake and also to achieve equal compliance, they were given an equivalent amount of whey protein. The study was conducted in the remission phase for the following reasons: (1) elimination of confounders like superimposed infection which may affect IP, (2) antibiotics, high dose coticosteroids used in the active phase, would have influenced the outcome, (3) difficulty in getting repeated biopsies during the acute phase, (4) non-compliance for oral intervention during the

active phase, (5) difficulty in maintaining a balance in the dietary intake of both groups in the acute phase, and (6) incomparability of the two groups due to variable CDAI score.

van der Hulst et al. [28] reported an unaltered LMR in the glutamine supplemented and increased LMR in the standard TPN group. The villous height also remained unchanged in the former, while there was a small decrease in the latter, thus suggesting preservation of IP and morphology by glutamine in a mixed group of 20 patients. In an uncontrolled study, Zoli et al. [27] described a significant improvement in LMR after supplementation of six grams of oral glutamine in 11 patients with inactive or moderate CD. However a detailed comparison is not possible as this study was published only as an abstract. Hond et al. [38] showed that a daily supplementation of 21 g of either oral glutamine or placebo for 4 weeks in 14 patients with CD did not normalize the altered IP to ^{51}CrEDTA. However, Akobeng et al. [39, 43] reported a comparable decrease in LMR in the glutamine-enriched and standard polymeric diet group in 16 children with active CD. Okenga et al. [40] did not show any effect of glutamine supplemented TPN on IP (lactulose/D-xylose ratio) in 14 patients with CD and four patients with UC. In a yet another study on eight healthy volunteers, Buchman et al. [44] showed an increase in LMR after 14 days of TPN. Followed by enteral refeeding, the LMR further increased significantly after refeeding with standard enteral formula compared to the glutamine and arginine supplemented enteral formula. Contrary to this the villous height and mucosal thickness increased significantly in the former compared to the latter. Nonetheless, the above-mentioned studies are inconclusive and limited in their impact and generalizability because of the following inadequacies: (a) heterogenous group of patients, (b) relatively small sample size, (c) lower doses of glutamine with short period of intervention, (d) variable routes for feeding, (e) lack of intestinal morphological evaluation, and (f) use of diverse methods for assessing IP.

In the present study we found a significant improvement in IP after glutamine supplementation. Recent molecular and protein chemistry studies have explained the role of glutamine in improving IP through various mechanisms [45]. A reduced level of intracellular glutamine is associated with a decrease in the transepithelial resistance of Caco-2 cell line [46]. Glutamine deprivation also decreases the insoluble fraction of claudin-1 and occludin proteins, but a partial reversibility of these "gate keeping" proteins is seen with glutamine supplementation [24]. Glutamine also prevents acetaldehyde induced injury to the TJ proteins [25] and regulates the integrity of the intercellular junction through the PI3-kinase/Akt pathway [47].

Interestingly, we also found an equal improvement in IP and morphology in the ACG which received whey protein. There are a few reports on whey protein or its constituents on IP [48, 49] and intestinal mucosa [50, 51]; however, the conclusions were unyielding. It is conceivable that a high protein diet might have played a role, as in both the GG and ACG protein intake increased by nearly 40%, to the target of 1.5 g/kg/day. Moreover, whey is a good source of all the amino acids which are reportedly absorbed more efficiently compared to free amino acid solutions [52]. Nevertheless, nutrition depletion and malnutrition per se have been found to play an important role in the impairment of IP and decrease of villous height [53].

In the present study a concomitant improvement in the intestinal morphology (VCR) was also seen. VCR is a marker of mucosal architecture and growth. There is evidence from a report in celiac disease that subtle changes in the jejunal morphometry have been correlated with IP [54]. Moreover, gluten withdrawal brings improvement in IP which precedes restoration of villous architecture in these patients [55]. Similar to our observation, van der Hulst et al. [28] have reported a preserved gut morphology with glutamine supplemented TPN; nonetheless, Buchman et al. [44] reported an increase in the VH of healthy volunteers refeeded with standard enteral formula as compared to glutamine supplemented formula. Glutamine has also been identified as a specific survival factor for the enterocytes and its decreased concentrations brings about a 12-fold induction in apoptosis and decrease in the cell number along the crypt villus axis [56]. It is a trophic nutrient for the small intestinal epithelia, and a preferred fuel for oxidative metabolism of the enterocytes [57, 58]. It has been recently reported that glutamine, arginine and leucine individually enhance restitution, proliferation, and migration of intestinal cells, thereby playing a vital role in the intestinal mucosal growth and function [59].

We observed a decrease in IELs, a marker of inflammation, in both the GG and ACG; although it was not significant. van der Hulst et al. [60] have also reported a significant decrease in the IELs after the administration of glutamine enriched TPN.

The plasma glutamine concentration remained unaltered after intervention in both the groups. Buchman et al. [44] have also reported a stable plasma glutamine concentration throughout the study in both glutamine and standard feeding groups. Ockenga et al. [40] too did not find any change in the plasma glutamine after glutamine enriched TPN. This is in contrast to the results from another study [28] where plasma glutamine increased from baseline in the glutamine enriched TPN group. The plasma glutamine levels are influenced by the inflammatory activity of the disease [61, 62], and the lack of acute inflammation in our patients might have been the reason for normal plasma

glutamine concentrations at baseline. A delay of 24–48 h in getting the post intervention blood samples coupled by a fast rate of glutamine uptake and utilization could be the probable reasons for the unaltered glutamine levels after supplementation in our patients.

Similar to the finding of Ockenga et al. [40] and Hond et al. [38], glutamine supplementation did not have any influence on the disease activity. Although, Akobeng et al. [43] reported an increase in the pediatric CDAI in children receiving glutamine enriched polymeric diet. In the present study nearly 50% of the patients in the GG had an increase in the stool frequency from 1–2 stools to 3–5 stools per day compared to the ACG. It is difficult to comment on the exact reason for this finding; however, it did not interfere in patient compliance.

In conclusion, this randomized controlled study showed a comparable effect of glutamine and whey protein in significantly improving IP and mucosal architecture. Thus, suggesting that glutamine is as effective as a high protein diet in improving IP in patients with CD.

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Conflict of interest There is no conflict of interest in this work.

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