Indian J Med Res 137, January 2013, pp 63-72

Pathogenesis of tropical sprue: A pilot study of antroduodenal manometry, duodenocaecal transit time & fat-induced ileal brake

Uday C. Ghoshal, Sunil Kumar, Asha Misra & Gourdas Choudhuri

Department of Gastroenterology Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Received November 2, 2011

***Background & objectives*: Small intestinal bacterial overgrowth (SIBO) due to ileal brake-induced hypomotility may cause tropical sprue (TS). We evaluated effect of infusion of fat or placebo in duodenum randomly in patients with TS and healthy controls on antroduodenal manometry (ADM) and mediators of ileal brake, and duodenocaecal transit time (DCTT).**

***Methods*: ADM and DCTT (lactulose hydrogen breath test, HBT) were evaluated with placebo and fat in eight controls and 13 patients with TS (diagnostic criteria: tests showing malabsorption of two unrelated substances, abnormal duodenal histology, absence of other causes, response to antibiotics and folate).**

***Results*: Patients with TS (6 had SIBO by glucose HBT) were similar in age and gender with controls. After fat infusion, proximal gut motility index (MI) was reduced compared to fasting state in TS, and DCTT was longer in TS than controls (200 min, 120-380 vs. 130, 70-160, *P*=0.001), though comparable after placebo (70 min, 30-140 vs. 60, 40-90). TS patients had higher PYY and neurotensin than controls after fat infusion. DCTT after fat infusion correlated with plasma level of PYY in TS but not in controls. Post-fat PYY and neurotensin levels were higher in TS with lower BMI (<16 kg/m^2) than those with higher BMI. Parameters of ileal brake (post-fat DCTT, PYY and neurotensin) were higher in patients with than without SIBO.**

***Interpretation & conclusions*: Fat infusion reduced proximal gut MI, increased DCTT, PYY, and neurotensin among patients with TS. Malabsorbed fat might cause exaggerated ileal brake reducing gut motility, promoting SIBO and bacterial colonization and malabsorption in TS.**

Key words Celiac disease - ileal brake - malabsorption syndrome - orocecal transit time - small intestine - tropical malabsorption

Tropical sprue (TS) is a common cause of malabsorption syndrome among adults in tropical countries including India[1]. TS is diagnosed by specific criteria, which include biochemical tests showing malabsorption of two unrelated substances, abnormal duodenal histology, absence of other causes of malabsorption and persistent response to antibiotics and folate[2,3]. Pathogenesis of this disease is unknown.

Bacterial infection has been proposed to cause this syndrome in view of small bowel bacterial colonization in most patients and overgrowth in a proportion, which responds to antibiotics[4]. In fact, frequent occurrence of small bowel bacterial colonization, overgrowth and predictable response to treatment with antibiotics might suggest that the name TS or tropical malabsorption is a misnomer[4]. It should rather be considered as a condition associated with small intestinal bacterial overgrowth (SIBO) and colonization in the tropics in absence of an anatomical cause. Therefore, there is a need to explore possible physiological factors responsible for SIBO in patients with TS. Normal fasting small intestinal motility, also called housekeeper activity, is an important factor preventing bacterial colonization of small bowel in health[5,6]. Conversely, conditions associated with poor intestinal motility are associated with bacterial colonization and overgrowth in the small bowel[6,7].

In two earlier studies, orocaecal transit time (OCTT) was found to be longer in patients with TS than in controls[4,8]. In one of these studies, OCTT correlated with degree of steatorrhoea[4]. Further, successful treatment of TS that led to normalization of faecal fat excretion also reduced OCTT[4]. Patients with SIBO with malabsorption are known to have longer OCTT than those without[9]. Therefore, we hypothesized that prolongation of OCTT in patients with TS might be related to inhibition of proximal small bowel motility by malabsorbed fat passing through the distal small intestine, which may cause SIBO. Such regulation of gastric and proximal small bowel motility by fat in the distal small bowel is called ileal brake, which is mediated by gut hormones such as peptide YY (PYY), neurotensin, and glucagon like peptide-1 (GLP-1)[10,11].

Prolonged OCTT in patients with TS might however, result from delayed gastric emptying or slowing of small bowel transit. In a previous study on patients with celiac disease, prolonged OCTT was found to result from delayed small intestinal transit rather than due to gastric emptying abnormality[12]. However, there is no study in patients with TS or SIBO. To obviate the possible influence of delayed gastric emptying on OCTT, we used a method to estimate duodenocaecal transit time (DCTT)[13,14]. The present study was aimed to evaluate *(i)* the effect of infusion of fat or placebo into small intestine randomly, in patients with TS as compared with healthy controls (HC) on alteration of parameters of antroduodenal manometry (ADM) and mediators of ileal brake (peptide YY, neurotensin and GLP-1) and on duodenocaecal transit time, and *(ii)* relationship between SIBO, parameters of ileal brake and degree of malnutrition.

Material & Methods

Patients: Thirteen consecutive patients with TS attending the Luminal Gastroenterology Clinic of Sanjay Gandhi Post Graduate Institute of Medical Sciences, a tertiary referral center in Lucknow, northern India, were subjected to evaluation of ADM and DCTT with placebo and fat. TS was diagnosed using standard criteria[15,16]. Eight HC were studied on a similar protocol. The study protocol was approved by the Institutional Ethics Committee and each subject gave written informed consent to take part in the study.

Outline of the study protocol: Fifty consecutive patients presenting with chronic small bowel diarrhoea during a 35 month period (from August 2006 to June 2009) were evaluated for biochemical evidence of malabsorption by urinary D-xylose test, faecal fat estimation and endoscopic duodenal biopsy. Patients with abnormal results in at least two of these three tests (urine D-xylose <0.8g/5g/5h, faecal fat >7g/day or faecal Sudan >10 droplet/hpf (high power field) and any degree of villous atrophy on duodenal histology) were investigated for cause of malabsorption using various tests such as, celiac disease by anti-endomysial antibody, anatomic abnormalities in the small bowel by barium study, associated acquired immunodeficiency syndrome by serology, giardiasis by stool microscopy and small intestinal biopsy, hypogammaglobulinaemia by serum immunoglobulin estimation and immunoproliferative small intestinal disease by small intestinal histology. TS was diagnosed using standard criteria that included; *(i)* biochemical evidence of malabsorption of two unrelated substances, *(ii)* histological abnormality on endoscopic duodenal biopsy, *(iii)* absence of another specific cause for malabsorption syndrome, and *(iv)* persistent response to treatment with antibiotics and folate. Of these 50 patients, 13 were diagnosed to have TS. Demographic, clinical, nutritional and laboratory parameters of patients were recorded in a standard proforma. At entry into the study, evidence of SIBO was sought using glucose hydrogen breath test (GHBT).

Glucose hydrogen breath test (GHBT): Glucose hydrogen breath test was performed using a breath gas analyzer (Bedfont gastrolyzer, Bedfont Scientific Ltd, ME13QX, England) with a standard protocol[4]. After an overnight fast, an average of four values was taken as the basal breath hydrogen level. Subjects were then

asked to take 100 g glucose dissolved in 200 ml water. Thereafter, breath hydrogen values were estimated every 15 min for the next 3 h. Persistent rise in breath hydrogen >12 ppm above basal (at least two readings) was considered diagnostic of SIBO[9]. Patients with high basal breath hydrogen levels were re-tested on another day after ensuring all the above precautions. No patient received antibiotics, proton pump inhibitor and drugs altering gut motility within one month before inclusion into the study.

Antroduodenal manometry (ADM): ADM was performed after an overnight fast using a water-perfusion system and a low-compliance polyvinyl catheter (Redtech, Calabasas, CA, USA). The catheter, with eight side holes placed 3 cm apart from each other, was passed through the nose without any sedation or anesthesia, over a guidewire, and was positioned under fluoroscopy such that at least two proximal ports were in the antrum and the remaining ports were in the duodenum. The guide wire was then removed.

Fasting motility was recorded for 2 h. At this point, 50 ml fat (20% w/v, Intravenous Fat Emulsion, Claris Life Sciences Limited, Ahmedabad, India) or placebo (normal saline) was infused through the central hole of the catheter into the duodenum, randomly. Manometry was recorded for 2 h following this. On the next day, subjects were crossed over to the other solution (coded) that may again be either fat or placebo after recording fasting manometry for 2 h. Manometry recording was continued over another 2 h following intra-duodenal infusion of fat or placebo (Fig. 1).

Analysis of antroduodenal manometry: The signal was visually scanned to remove artifacts (*e.g.*, those due to cough), filtered (9 and 24/ min for antral and duodenal frequency limits, respectively), and then analyzed by a single investigator, who was not aware of the code of the solution (fat or placebo), using GiPC motility software (Redtech, USA). In the fasting record, presence of spontaneous migratory motor complex and its duration was looked for. Frequency, amplitude and duration of migratory motor complex in fasting state were calculated by standard technique. Post fat/ placebo infusion record was evaluated for conversion from fasting to fed pattern. Motility index (MI) was calculated by computer software by the formula: MI = Log_{10} (number of peaks x sum of amplitude + 1). The same parameters were analyzed for the manometry record after infusion of fat or placebo.

Duodenocecal transit time (DCTT): DCTT was estimated using a hydrogen breath gas analyzer (Bedfont gastrolyzer, Bedfont Scientific Ltd, ME13QX, England) during ADM. Basal breath specimens were obtained after an overnight fast; the subjects were asked to avoid slowly absorbed carbohydrates (bread, potato, corn) and fibers the previous evening to avoid delayed excretion of hydrogen in breath[4]. Tea, coffee, cigarette smoking and physical exercise were not allowed for 2 h before and during the test so as to avoid interference of breath hydrogen content[4]. The subjects brushed their teeth properly, rinsed mouth with antiseptic mouth wash followed by tap water to eliminate the possibility of an early hydrogen peak due to action of oral bacteria on test sugars. An average of

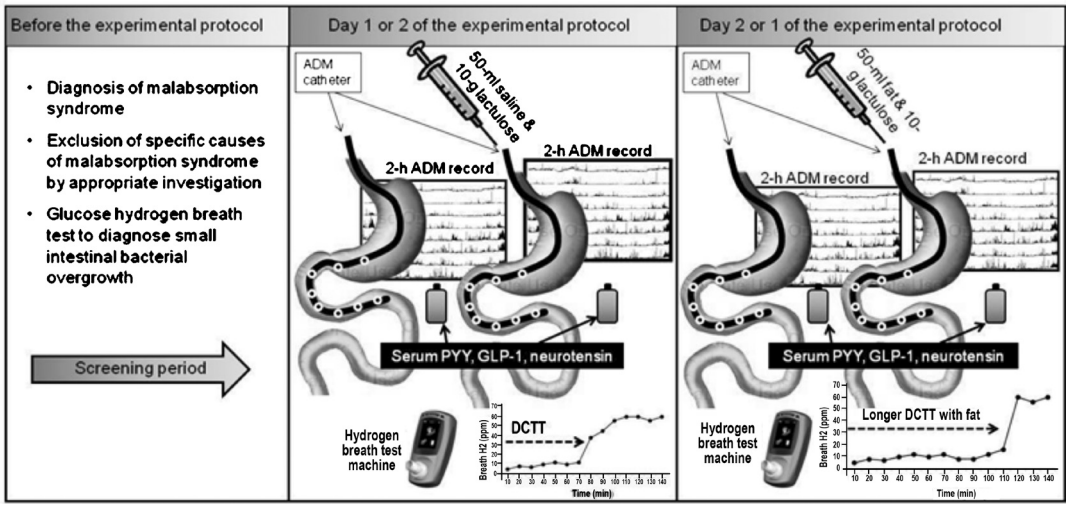

Fig. 1. Outline of the protocol used in this study; ADM, antroduodenal manometry; PYY, peptide YY; GLP-1, glucagon like peptide-1; DCTT, duodenocaecal transit time.

three values was taken as the fasting breath hydrogen level. Subsequently, breath hydrogen was estimated every 10 min for 3 h after administration of 15 ml lactulose along with fat or placebo solution through the central port of manometry catheter into the duodenum. Lactulose hydrogen breath test was continued for 4-5 h if no peak was obtained. Increase in hydrogen excretion (ppm) following lactulose administration was calculated by subtracting the fasting value from the highest value of hydrogen excretion obtained. The time interval between lactulose administration and rise of breath hydrogen by 20 ppm above the fasting level was considered as DCTT. Duodenocaecal transit time estimation after infusion of fat or placebo (coded solutions given randomly) into small bowel was done again on the second day (Fig. 1).

Estimation of peptide YY, neurotensin and glucagon like peptide-1(GLP-1): Blood sample (10 ml) was collected by venupuncture in EDTA and aprotinin before giving fat or placebo (baseline) and after 1 h of fat or placebo administration into the small bowel on both the days during ADM and DCTT in patients with TS and controls. Two samples (baseline and after 1 h of fat or placebo administration) on each day were taken. Plasma was separated and stored at -40^{o} C. Peptide YY, neurotensin and GLP-1 levels were estimated from plasma by commercially available ELISA Kits (Peninsula Laboratories Inc, United Kingdom) (Fig. 1).

Treatment and follow up: All patients were treated with tetracycline (500 mg thrice daily for one month followed by 500 mg twice daily for another month) and folic acid (10 mg/day) for at least 6 months. At least 2 wk after stopping treatment with tetracycline, 72 h faecal fat, D-xylose, histological examination of endoscopic duodenal biopsy, and glucose hydrogen breath tests were repeated.

Statistical analysis: Data were analyzed using SPSS (Version 15.0; The Predictive analytics company, SPSS Incl. Chicago), and Epi Info™ (Version 6.0; A database and statistics program for public health professionals, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, 2002). Continuous data were expressed as median and range. Differences between continuous and categorical variables were analyzed using Mann-Whitney U-test and Chi-square test with Yates' correction as applicable, respectively. Paired data were analyzed using Wilcoxon sign rank test. Follow up data were analyzed using Mann-Whitney U-test at each time point.

Results

Demographic, clinical and laboratory parameters: Of the 50 patients with chronic small bowel diarrhoea, 13 (26%) with TS were included in the study. Patients with TS were comparable in age [35 yr, (24-62) vs. 35 yr, (17-54)] and gender [male 7/13 (54%) vs. 6/8 (75%)] with HC. Demographic, clinical and laboratory parameters of patients with TS before and after treatment and HC are summarized in Table I. Median body mass index (BMI) of patients with TS and HC was 15.8 kg/m^2 (range 12.8 to 18.8 kg/m^2) and 23.5 kg/m^2 (range 21.5 to 24.8 kg/m^2), respectively.

Antroduodenal manometry: Both fat and saline infusion resulted in disappearance of spontaneous migratory motor complex in most patients with TS and HC. Duodenal MI in patients with TS and HC was not significantly different after fat infusion. Fat infusion led to reduction in duodenal MI both in patients with TS and HC. Though placebo infusion reduced duodenal MI in patients with TS, it was less marked in HC (Table II).

Result of peptide YY, neurotensin and GLP-1: Plasma peptide YY and neurotensin levels were higher in patients with TS compared to HC after infusion of fat. However, there was no difference in plasma levels of GLP-1 in patients with TS and HC after fat infusion. Post-placebo plasma peptide YY, neurotensin and GLP-1 in patients with TS were comparable to those in HC (Fig. 2).

In patients with TS, post-fat plasma peptide YY and neurotensin levels were higher in subjects with lower BMI (<16 kg/m^2, n=7)[17] than those with higher BMI (>16 kg/m^2, n=6) (median 31.4 pg/ml, range 21.6-35.6 vs. 19.9 pg/ml, range 11.7-32.4, *P*=0.02) and (39.1 pg/ml, range 11.5-44.1 vs. 17.1 pg/ml, range 10.4-25.4, *P*=0.022), respectively. There was no difference in the plasma level of GLP-1 among these two groups.

Duodenocecal transit time (DCTT): Post-placebo DCTT was comparable among patients with TS and HC (70 min, 30-140 vs. 60 min, 40-90). Post-fat DCTT was longer compared to post-placebo in patients with TS (200 min, 120-380 vs. 70 min, 30-140, *P*=0.003) and HC (130 min, 70-160 vs. 60 min, 40-90, *P*=0.027) [Fig. 3 (A), (B) and (C)]. However, post-fat DCTT was longer in patients with TS as compared to HC (200 min, 120-380 vs. 130 min, 70-160, *P*=0.001).

Post-fat DCTT was longer in subjects with lower BMI (<16 kg/m^2) compared to those with higher BMI

Table I. Demographic, clinical and laboratory parameters of patients with tropical sprue (TS) before and after treatment and healthy controls (HC)

Parameters	Patients with TS (N=13)		HC (N=8)	*P* value
	Before treatment	After treatment		
Age (yr)	35 (24-62)	-	35 (17-54)	NS
M : F	7 : 6	-	6 : 2	NS
Stool frequency (number/day)	10 (4-20)	1 (1-3)	-	0.001
Haemoglobin (g/dl)	11.6 (3.7-13.6)	12.8 (10.6-15.1)	-	0.001
Serum albumin	3 (2-3.9)	3.5 (2.3-5)	-	0.051
Faecal fat (g/day)	9.8 (6.1-26)	4 (3.1-6.3)	-	0.018
Faecal Sudan (droplets/HPF)	14 (4-26)	6 (3-10)	-	0.027
D-xylose (g/5g/5h)	0.59 (0.28-0.94)	1.08 (0.17-2.1)	-	0.015
No malnutrition n (%) (BMI 18.1 to 25 kg/m^2)	3 (23.1)	6 (46.1)	8 (100%)	0.002
Mild malnutrition n (%) (BMI 16 to 18 kg/m^2)	3 (23.1)	4 (30.8)	0	0.22
Severe malnutrition n (%) (BMI less than 16 kg/m^2)	7 (53.8)	3 (23.1)*	0	0.025
Positive GHBT n (%)	6 (46)	1 (8)	-	0.03
Partial villous atrophy n (%)	7 (54)	1 (8)	-	0.03
Subtotal villous atrophy n (%)	1 (8)	0	-	-
Increased mononuclear infiltrate with increased intra-epithelial lymphocytes n (%)	5 (38)	6 (46)	-	NS
All three tests abnormal (urine D-xylose, faecal fat or faecal Sudan and abnormal villi on duodenal histology) n (%)	12 (92)	0	-	-
Two test abnormal (faecal fat and abnormal villi on duodenal histology) n (%)	1 (8)	0	-	-

All the continuous data are presented as median and range and categorical data as number and percentages. The continuous and categorical data were analyzed using Wilcoxon sign rank test, Mann-Whitney U test and chi-square test, respectively
NS, not significant; M, male; F, female; BMI, body mass index; HPF, high power field; GHBT, glucose hydrogen breath test. Data on malnutrition presented here are at 4 month follow up. No patient had malnutrition at 8 month follow up

(>16 kg/m^2) (265 min, range 200-380 min vs. 170 min, range 120-190 min, *P*<0.004). However, post-placebo DCTT was comparable among subjects with lower BMI (<16 kg/m^2) than those with higher BMI (>16 kg/m^2) (70 min, range 70-140 min vs. 60 min, range 30-90 min, *P*=0.093).

Comparison of the parameters between patients with TS with and without SIBO: Of the 13 patients with TS, six had SIBO on GHBT. MI of duodenum in patients with TS with SIBO was lower after fat infusion than in those without SIBO. However, before infusion of fat, duodenal MI of patients with TS with and without SIBO was somewhat comparable (Table III). Average MI of four proximal duodenal ports (median 21 mmHg, range 12-30 mmHg vs. median 13 mmHg, range 10-15 mmHg, *P*=0.028) and four distal ports (median 20 mmHg, range 13-28 mmHg vs. median 12 mmHg, range 11-16 mmHg, *P*=0.046) were higher before compared to after fat infusion in patients with TS with SIBO. However, MI of four proximal duodenal (median 23 mmHg, range 15-35 mmHg vs. median 22 mmHg, range 16-38 mmHg) and four distal ports (median 24 mmHg, range 16-33 mmHg vs. median 21 mmHg, range 18-38 mmHg) was comparable to before than after fat infusion in patients with TS without SIBO.

Post-fat DCTT was longer in patients with TS with SIBO as compared to those without SIBO (275 min, range 190-365 min vs. 165 min, range 125-180 min, *P*=0.004), though it was comparable after placebo

Table II. Comparison of antroduodenal manometry parameters in patients with tropical sprue (TS) and healthy controls (HC)

Parameters	HC (N=8)			TS (N=13)		
	Before placebo	After placebo	*P* value	Before placebo	After placebo	*P* value
Recording time (min)	127 (122-140)	122 (110-131)	ns	132 (121-143)	118 (110-133)	0.028
Presence of spontaneous MMC	7/8	0/8	0.001	9/13	1/13	0.004
Spontaneous MMC duration (min)	6 (3-8)	0	-	8 (4-17)	12	-
Motility index (mmHg)						
Port-1	24 (18-37)	20 (13-24)	ns	25 (16-59)	21 (14-37)	0.004
Port-2	22 (16-33)	20 (15-31)	ns	24 (11-35)	14 (10-37)	0.033
Port-3	23 (20-30)	20 (14-29)	0.06	33 (16-65)	29 (12-69)	0.041
Port-4	29 (21-48)	30 (17-41)	ns	30 (23-59)	26 (17-40)	0.021
Port-5	29 (21-37)	23 (16-32)	ns	24 (20-53)	21 (14-40)	0.006
Port-6	32 (18-48)	28 (15-34)	0.01	42 (29-74)	30 (17-62)	0.003
Port-7	28.(18-42)	24 (15-27)	0.04	30 (25-36)	23 (12-40)	0.008
Port-8	24 (7-27)	21 (7-28)	ns	26 (16-38)	18 (13-37)	0.041
Parameters	HC (N=8)			TS (N=13)		
	Before fat	After fat	*P* value	Before fat	After fat	*P* value
Recording time (min)	127 (120-130)	123 (115-129)	0.08	129 (117-137)	124 (111-131)	NS
Presence of spontaneous MMC	8/8	2/8	0.006	13/13	3/13	0.002
Spontaneous MMC duration (min)	8 (3-9)	8.5 (6-11)	0.65	9 (2-12)	11 (8-15)	NS
Motility index (mmHg)						
Port-1	22 (19-40)	17 (11-25)	0.05	22 (16-35)	16 (10-51)	0.075
Port-2	27 (17-40)	17 (13-34)	0.036	23 (14-30)	15 (10-23)	0.001
Port-3	30 (21-38)	23 (13-30)	0.025	27 (18-42)	21 (9-27)	0.001
Port-4	33 (23-52)	19 (16-35)	0.017	33 (19-63)	23 (16-53)	0.002
Port-5	33 (24-159)	22 (14-125)	0.025	27 (19-51)	22 (10-45)	0.013
Port-6	36 (19-50)	27 (15-35)	0.012	31 (22-57)	21 (16-37)	0.002
Port-7	31 (18-42)	23.2 (13-29)	0.036	31 (16-36)	25 (15-31)	0.023
Port-8	26 (8-38)	22 (8-30)	0.069	22 (17-37)	20 (15-29)	0.013

All the continuous data are presented in median and range (value within parenthesis). The continuous and categorical data were analyzed using Wilcoxon sign rank test and chi-square test, respectively. MMC, motor migratory complex; NS, not significant. Difference in parameters of motility (occurrence of MMC, its duration and MI) was significant neither after fat or placebo among patients and HC

infusion (65 min, range 60-130 min vs. 60 min, range 40-95 min). Plasma levels of peptide YY (*P*=0.015) and neurotensin (*P*=0.001) were higher among patients with SIBO than those without SIBO. However, there was no difference in plasma levels of GLP-1 among these two groups (Fig. 4).

Correlation between plasma peptide YY, neurotensin, GLP-1 and DCTT: Post-fat DCTT correlated with plasma level of peptide YY in patients with TS (Spearman rho = 0.61, *P*=0.04) but not in HC (Spearman rho= 0.58, *P*=0.17). However, plasma level of neurotensin and GLP-1 did not correlate with DCTT either in patients with TS or HC.

Results of treatment and follow up: All patients with TS responded to the treatment as reflected by reduction in diarrhoea, increase in body weight and improvement in

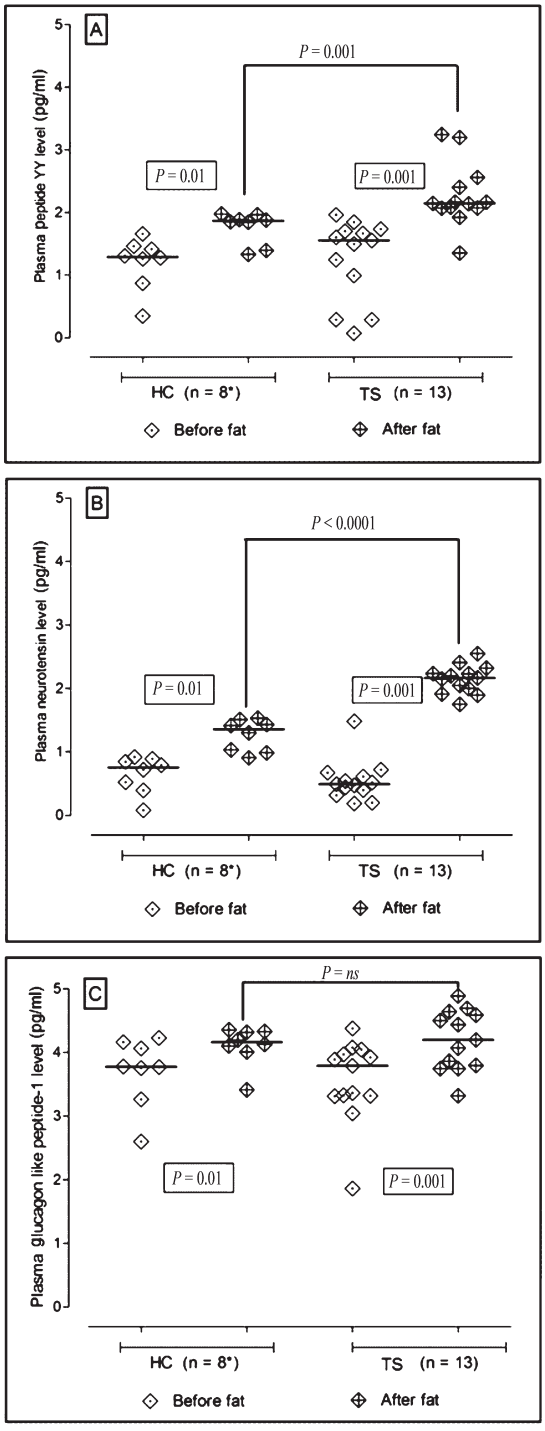

Fig. 2. Comparison of plasma level of; **(A)** peptide YY before and after infusion of fat and placebo in patients with TS and HC; **(B)** neurotensin before and after infusion of fat and placebo in patients with TS and HC; **(C)** GLP-1 before and after infusion of fat and placebo in patients with TS and HC. (*) shows that one HC plasma level of GLP-1 could not be measured as the patient declined; the unpaired and paired data were analyzed using Mann-Whitney U test and Wilcoxon sign rank test, respectively. TS, tropical sprue; HC, healthy controls; GLP-1, glucagon like peptide-1.

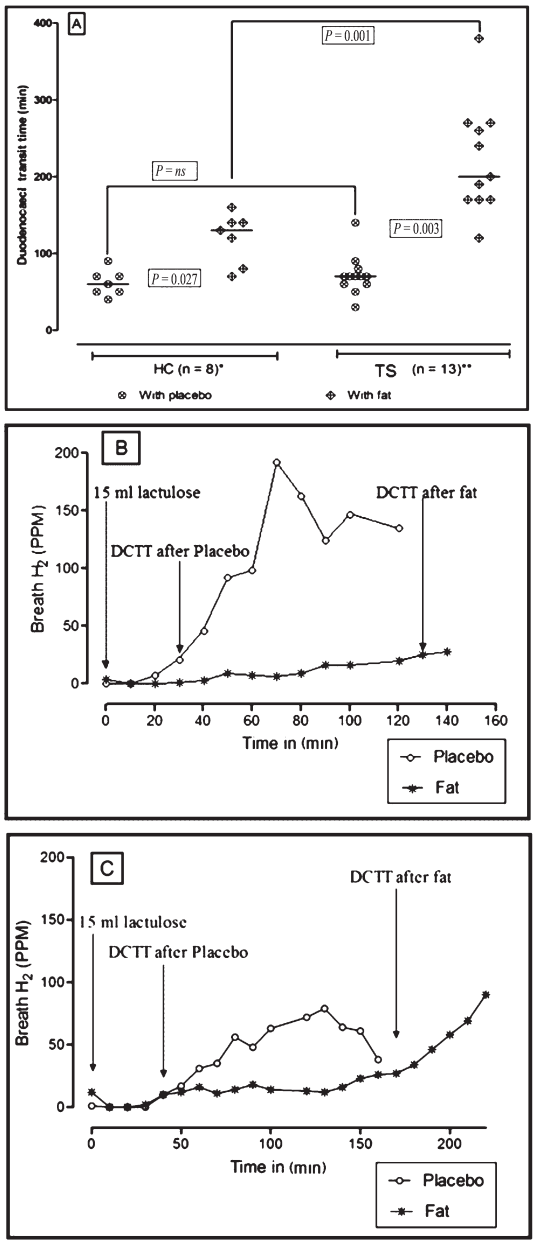

Fig. 3. Comparison of **(A)** duodenocaecal transit time in patients with TS and HC after placebo and fat and a representative picture of duodenocecal transit time **(B)** in HC and **(C)** in patients with TS both after infusion of placebo or fat into small intestine, respectively. TS, tropical sprue; HC, healthy controls; DCTT, duodenocaecal transit time; PPM, parts per million, the unpaired and paired data were analyzed using Mann-Whitney U test and Wilcoxon sign rank test, respectively. Note: (*) shows that one HC did not undergo the test and another patients in whom after fat DCTT could not be measured due to high basal rate. (**) shows that in one patient with TS, DCTT could not be measured as the basal breath hydrogen was higher (>20 ppm) and in another one the lactulose hydrogen breath test after infusion of fat was measured till 360 min but there was no increase in breath hydrogen above basal hence, DCTT could not be measured in this patient.

Table III. Comparison of motility parameters among patients with tropical sprue (TS) with and without small intestinal bacterial overgrowth (SIBO) before and after fat infusion

Parameters	Patients with TS (Before fat MI)			Patients with TS (After fat MI)		
	With SIBO (N=6)	Without SIBO (N=7)	*P* value	With SIBO (N=6)	Without SIBO (N=7)	*P* value
Recording time (min)	131 (117-133)	126 (117-137)	NS	124 (113-130)	124 (111-131)	NS
Presence of spontaneous MMC	6/6	7/7	NS	2/6	1/7	NS
Spontaneous MMC duration (min)	10 (3-12)	9 (2-12)	NS	9 (8-11)	15	NS
Motility index (mmHg)						
Port-1	19 (12-26)	21 (17-35)	NS	13 (10-13)	19 (15-51)	0.001
Port-2	18 (10-29)	22 (14-28)	NS	13 (11-17)	18 (10-24)	0.073
Port-3	23 (14-33)	27 (22-34)	NS	12 (10-16)	22 (21-27)	0.001
Port-4	23 (14-32)	35 (21-64)	0.022	15 (13-18)	27 (16-53)	0.005
Port-5	20 (15-30)	28 (24-44)	0.022	12 (10-15)	23 (22-45)	0.001
Port-6	22 (13-34)	29 (24-57)	0.035	15 (11-17)	21 (18-36)	0.001
Port-7	23 (13-31)	28 (20-35)	NS	14 (11-20)	24 (18-29)	0.005
Port-8	17 (12-22)	22 (17-37)	0.073	14 (13-18)	20 (15-29)	0.022

All the continuous data are presented in median and range (value within parenthesis). The continuous and categorical data were analyzed using Mann-Whitney U test and chi-square test, respectively. MMC, migratory motor complex; ns, not significant; MI, motility index

haematological, biochemical parameters and tests for malabsorption (Table I). Though patients with SIBO had BMI comparable to those without SIBO at presentation (15.8 kg/m^2, range 12.8-15.8 vs. 17.2, range 15.2-18.8 kg/m^2), treatment with tetracycline resulted in greater weight gain among patients with than those without SIBO (at 4-mo, 20.6 kg/m^2, range 16.0-25.6 vs. 17.3, range 16.6-23.0 kg/m^2, *P*=0.03, and 6 month, 22.7 kg/m^2, range 19.7-26.9 vs. 18.2, range 17.3-24.6 kg/m^2, *P*<0.001). However, at 8 month follow up, BMI of the two groups was comparable (23.8 kg/m^2, range 20.8-28.6 vs. 23.3, range 21.3-26.7 kg/m^2).

Discussion

The present study showed that infusion of fat into the proximal gut resulted in *(i)* reduction of MI, *(ii)* prolongation of DCTT particularly among patients with TS, *(iii)* reduction in gut motility as shown by prolongation of DCTT and increase in the plasma levels of peptide YY and neurotensin, *(iv)* DCTT following fat infusion in patients with TS correlated with plasma levels of peptide YY, *(v)* patients with

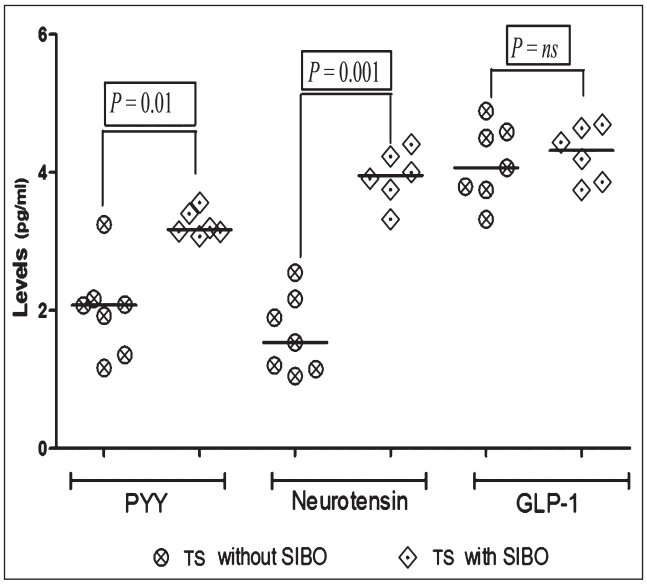

Fig. 4. Comparison of plasma levels of peptide YY, neurotensin and GLP-1 after infusion of fat in patients with TS with and without SIBO, Data were analyzed using Mann-Whitney U test. TS, tropical sprue; HC, healthy controls; GLP-1, glucagon like peptide-1; SIBO, small intestinal bacterial overgrowth.

severe malnutrition (BMI <16 kg/m^2)[17] due to TS had higher post-fat plasma PYY, neurotensin levels and longer DCTT than those with less severe malnutrition, *(vi)* patients with TS associated with SIBO had reduced post-fat MI, longer DCTT, higher plasma levels of PYY and neurotensin, and *(vii)* antibiotic treatment caused greater increase in BMI on follow up in patients with SIBO.

Though celiac disease is the commonest cause of malabsorption syndrome in temperate countries[18-20], it constitutes only 10-30 per cent of adult patients in the tropics[1, 21]. On the contrary, TS is the commonest cause of malabsorption syndrome in many parts on India[1], though one study from northern India showed celiac disease to be the commonest cause[22]. TS is diagnosed by specific criteria[2,3] and has also been called as "idiopathic malabsorption of tropics", "tropical malabsorption" and "post-infective tropical malabsorption"[23-25]. An infective cause has been suspected in the pathogenesis because of occurrence of TS in areas with poor hygiene, demonstration of SIBO or small intestinal colonization[4] and response to treatment with antibiotics[4].

What could be the reasons for small intestinal bacterial colonization and overgrowth in patients with TS? Patients having SIBO with malabsorption are known to have longer OCTT than those without[9]. OCTT is prolonged in patients with TS causing stasis and SIBO[4,8]. In the present study 46 per cent of patients with TS had SIBO using GHBT. The sensitivity and specificity of GHBT are 44 and 80 per cent to diagnose SIBO (>10^5 cfu/ml bacteria in jejunal aspirate)[9,26]. Low sensitivity of GHBT is a limitation of the study. Though bacterial colonization lesser than 10^5 cfu/ml would not be detected by GHBT, it is frequent in TS and is important in pathogenesis[4].

We previously showed that prolonged OCTT in patients with TS correlated with steatorrhoea[4]. Similar observations have been reported in celiac disease[12]. In patients with TS, successful treatment with antibiotics resulted in reduction in steatorrhoea and OCTT. Therefore, we proposed that malabsorbed fat in patients with TS induced ileal brake resulting in prolonged OCTT[4]. It is also possible that patients with TS have exaggerated ileal brake. Ileal brake results from liberation of gut hormones like peptide YY, neurotensin and GLP-1[27,28].

The current study showed that malabsorbed fat in patients with TS could cause exaggerated ileal brake, which explains prolonged OCTT with resultant stasis causing bacterial colonization and overgrowth. Though SIBO and bacterial colonization may be important for initiation and continuation of malabsorption of nutrients, these may also result from malabsorption of fat which liberates PYY and neutrofensin.

Our finding showing patients with severe malnutrition having higher post-fat plasma PYY, neurotensin levels and longer DCTT than those with less severe malnutrition suggests that exaggerated ileal brake could have led to greater malabsorption and malnutrition.

Reduction of MI following saline infusion into gut, particularly among healthy controls, might be related to lactulose infusion rather than saline infusion as has been reported previously with other oligosaccharides[13,29,30]. Two hours recording period may be short to study migratory motor complex. However, short recording time would not pose limitation for studying other parameters particularly comparing data before and after an intervention. Small sample size, particularly for subgroup analysis is another limitation of this study.

In conclusion, the present study shows that patients with TS had lower MI in proximal duodenal port, infusion of fat into the upper gut resulted in further reduction of MI, fat infusion was also associated with prolongation of DCTT particularly among patients with TS, reduction in proximal gut motility as shown by DCTT following fat infusion was associated with increase in the plasma levels of peptide YY, neurotensin and GLP-1, and DCTT following fat infusion in patients with TS correlated with plasma levels of peptide YY.

Acknowledgment

Authors thank all the patients and volunteers for taking part in this study, and Shri Raghunath of the GI pathophysiology and motility laboratory for his help and support in antroduodenal manometry test. This work was supported by an extramural research grants from the Department of Science and Technology (no. SR/SO/HS-24/2003), Government of India, to the first author (UCG). The second author (SK) thanks the Indian Council of Medical Research (ICMR), New Delhi, for providing his SRF fellowship.

References

1. Ghoshal UC, Mehrotra M, Kumar S, Ghoshal U, Krishnani N, Misra A, *et al*. Spectrum of malabsorption syndrome among adults & factors differentiating celiac disease & tropical malabsorption. *Indian J Med Res* 2012; *136* : 451-9.
2. Klipstein FA. Tropical sprue. In: Sleisenger MH, Fordtran JS, Scharschmidt BF, Feldman M, editors. *Gastrointestinal and liver disease. Pathophysiology, diagnosis, management*. Philadelphia: W.B. Saunders; 1993. p. 1097-105.

3. Farthing MJG. Tropical malabsorption and tropical diarrhea. In: Feldman M, Scharschmidt BF, Sleisenger MH, editors. *Gastrointestinal and liver disease. Pathophysiology, diagnosis, management*. Philadelphia: W.B. Saunders 1998. p. 1575-84.
4. Ghoshal UC, Ghoshal U, Ayyagari A, Ranjan P, Krishnani N, Misra A, *et al*. Tropical sprue is associated with contamination of small bowel with aerobic bacteria and reversible prolongation of orocecal transit time. *J Gastroenterol Hepatol* 2003; *18* : 540-7.
5. Stotzer PO, Bjornsson ES, Abrahamsson H. Interdigestive and postprandial motility in small-intestinal bacterial overgrowth. *Scand J Gastroenterol* 1996; *31* : 875-80.
6. Quigley EM. Microflora modulation of motility. *J Neurogastroenterol Motil* 2011; *17* : 140-7.
7. Pimentel M, Soffer EE, Chow EJ, Kong Y, Lin HC. Lower frequency of MMC is found in IBS subjects with abnormal lactulose breath test, suggesting bacterial overgrowth. *Dig Dis Sci* 2002; *47* : 2639-43.
8. Cook GC. Delayed small-intestinal transit in tropical malabsorption. *BMJ* 1978; *2* : 238-40.
9. Ghoshal UC, Ghoshal U, Das K, Misra A. Utility of hydrogen breath tests in diagnosis of small intestinal bacterial overgrowth in malabsorption syndrome and its relationship with oro-cecal transit time. *Indian J Gastroenterol* 2006; *25* : 6-10.
10. Maljaars J, Romeyn EA, Haddeman E, Peters HP, Masclee AA. Effect of fat saturation on satiety, hormone release, and food intake. *Am J Clin Nutr* 2009; *89* : 1019-24.
11. Pironi L, Stanghellini V, Miglioli M, Corinaldesi R, De Giorgio R, Ruggeri E, *et al*. Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY. *Gastroenterology* 1993; *105* : 733-9.
12. Spiller RC, Lee YC, Edge C, Ralphs DN, Stewart JS, Bloom SR, *et al*. Delayed mouth-caecum transit of a lactulose labelled liquid test meal in patients with steatorrhoea caused by partially treated coeliac disease. *Gut* 1987; *28* : 1275-82.
13. Gielkens HA, van den Biggelaar A, Vecht J, Onkenhout W, Lamers CB, Masclee AA. Effect of intravenous amino acids on interdigestive antroduodenal motility and small bowel transit time. *Gut* 1999; *44* : 240-5.
14. Verkijk M, Vecht J, Gielkens HA, Lamers CB, Masclee AA. Effects of medium-chain and long-chain triglycerides on antroduodenal motility and small bowel transit time in man. *Dig Dis Sci* 1997; *42* : 1933-9.
15. Baker SJ, Mathan VI. Syndrome of tropical sprue in South India. *Am J Clin Nutr* 1968; *21* : 984-93.
16. Baker SJ, Mathan VI. *Tropical sprue in Southern India. Wellcome Trust Collaboration Study 1961-1969*. London: Churchill Livingstone; 1971. p. 453.
17. Chen X, Wang Y. Optimal body mass index cut points. *Int J Epidemiol* 2010; *39* : 1045-7.
18. Barada K, Bitar A, Mokadem MA, Hashash JG, Green P. Celiac disease in Middle Eastern and North African countries: a new burden? *World J Gastroenterol* 2010; *16* : 1449-57.
19. Ozdemir Z, Jensen ON, Malchow-Moller A. [Celiac disease in adults]. *Ugeskr Laeger* 2000; *162* : 643-8.
20. Catassi C, Fabiani E. The spectrum of coeliac disease in children. *Baillieres Clin Gastroenterol* 1997; *11* : 485-507.
21. Ranjan P, Ghoshal UC, Aggarwal R, Pandey R, Misra A, Naik S, *et al*. Etiological spectrum of sporadic malabsorption syndrome in northern Indian adults at a tertiary hospital. *Indian J Gastroenterol* 2004; *23* : 94-8.
22. Yadav P, Das P, Mirdha BR, Gupta SD, Bhatnagar S, Pandey RM, *et al*. Current spectrum of malabsorption syndrome in adults in India. *Indian J Gastroenterol* 2011; *30* : 22-8.
23. Cook GC. Aetiology and pathogenesis of postinfective tropical malabsorption (tropical sprue). *Lancet* 1984; *1* : 721-3.
24. Montgomery RD, Shearer AC. The cell population of the upper jejunal mucosa in tropical sprue and postinfective malabsorption. *Gut* 1974; *15* : 387-91.
25. Kumar S, Ghoshal UC, Jayalakshmi K, Roy R, Misra A, Khetrapal CL. Abnormal small intestinal permeability in patients with idiopathic malabsorption in tropics (tropical sprue) does not change even after successful treatment. *Dig Dis Sci* 2011; *56* : 161-9.
26. Gwee KA. The hydrogen breath test: new uses for an old test. *J Neurogastroenterol Motil* 2010; *16* : 228-9.
27. Van Citters GW, Lin HC. Ileal brake: neuropeptidergic control of intestinal transit. *Curr Gastroenterol Rep* 2006; *8* : 367-73.
28. Dumoulin V, Moro F, Barcelo A, Dakka T, Cuber JC. Peptide YY, glucagon-like peptide-1, and neurotensin responses to luminal factors in the isolated vascularly perfused rat ileum. *Endocrinology* 1998; *139* : 3780-6.
29. Stevens JE, Doran S, Russo A, O'Donovan D, Feinle-Bisset C, Rayner CK, *et al*. Effects of intravenous fructose on gastric emptying and antropyloroduodenal motility in healthy subjects. *Am J Physiol Gastrointest Liver Physiol* 2009; *297* : G1274-80.
30. Washington N, Harris M, Mussellwhite A, Spiller RC. Moderation of lactulose-induced diarrhea by psyllium: effects on motility and fermentation. *Am J Clin Nutr* 1998; *67* : 317-21.

Reprint requests: Dr Uday C. Ghoshal, Additional Professor, Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226 014, India
e-mail: udayghoshal@gmail.com