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Postprandial platelet-poor plasma 5-hydroxytryptamine concentrations during diarrhea and constipation periods of alternating-type irritable bowel syndrome patients

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Background/aims: *Our aim was to measure concentrations of platelet-poor plasma 5-hydroxytryptamine and to assess any relationship with gastrointestinal symptomatology under fasting and fed conditions in alternating-type irritable bowel syndrome during both constipation and diarrhea periods separately. Results of the two periods were compared with each other as well as with the results of the controls.* ***Methods:*** *Nine patients with alternating diarrhea and constipation symptoms and 9 controls were enrolled. Serial plasma 5-hydroxytryptamine was measured for 1 hour under fasting and for 3 hour after a standard carbohydrate meal. Patients underwent the same measurements during constipation and diarrhea periods separately. Serum 5-hydroxytryptamine concentrations were determined by high-performance liquid chromatography. Symptomatology was assessed throughout the study.* ***Results:*** *Patients exhibited higher concentrations of platelet-poor plasma 5-hydroxytryptamine under fed conditions during diarrhea, especially at postprandial 30 minutes ($p<0.05$) compared with concentrations during constipation. Increases in postprandial plasma 5-hydroxytryptamine concentrations relative to fasting concentrations were also significantly higher during the diarrhea period than during constipation and in controls ($p<0.05$). Although there was no significant correlation between plasma 5-hydroxytryptamine concentrations and symptom scores, patients had worse postprandial symptomatology during diarrhea compared with controls ($p<0.05$).* ***Conclusions:*** *Platelet-poor plasma 5-hydroxytryptamine concentrations after meal ingestion differ between constipation and diarrhea periods in alternating-type irritable bowel syndrome. Postprandial symptomatology is also more prominent during diarrhea. These results suggest that differences in plasma levels of serotonin between diarrhea and constipation may underlie the pathogenesis of alternating-type irritable bowel syndrome and could be involved in some aspects of symptomatology.*

Key words: Alternating-type irritable bowel syndrome, 5-hydroxytryptamine, bloating, pain, urgency

Alterne tip irritabl barsak sendromlu hastaların ishal ve kabızlık dönemlerinde yemek sonrası trombositten fakir plazmada 5-hidroksitriptamin konsantrasyonları

Amaç: *Amacımız alterne tip irritabl barsak sendromunda hem kabızlık hem de ishal dönemlerinde açlık ve tokluk sırasında trombositten fakir plazmada 5-hidroksitriptamin konsantrasyonlarını ölçmek ve bunun gastrointestinal semptomatoloji ile ilişkisini incelemek ve iki dönemdeki sonuçları birbiri ile ve kontrollerle karşılaştırmaktı.* ***Yöntem:*** *Dokuz tane ishal ve kabızlık semptomları alterne eden hasta ve dokuz kontrol çalışmaya alındı. Plazma 5-hidroksitriptamin düzeyleri yemekten bir saat önce açlıkta ve karbohidrattan zengin standart bir yemekten sonra 3 saat boyunca ölçüldü. Hastalara aynı ölçümler kabızlık ve ishal dönemlerinde olmak üzere 2 kez yapıldı. Serum 5-hidroksitriptamin konsantrasyonları "high-performance liquid chromatography" tekniği kullanılarak ölçüldü. Çalışma boyunca semptomlar değerlendirildi.* ***Bulgular:*** *Hastalar, kabızlık dönemlerine göre ishal dönemlerinde özellikle postprandial 30. dakika başta olmak üzere yemek sonrası daha yüksek trombositten fakir plazmada 5-hidroksitriptamin konsantrasyonlarına sahipti ($p<0.05$). Açlığa göre postprandial plazma 5-hidroksitriptamin konsantrasyonlarındaki artış da kabızlık dönemleri ve kontrollerdekine göre belirgin olarak daha yüksek bulundu ($p<0.05$). Her ne kadar plazma 5-hidroksitriptamin konsantrasyonları ile semptom skorları arasında anlamlı bir ilişki bulunamasa da, hastaların ishal dönemlerinde postprandial semptomatolojileri kontrollerinkine göre daha kötü bulundu ($p<0.05$).* ***Sonuç:*** *Trombositten fakir plazmada 5-hidroksitriptamin konsantrasyonları alterne tip irritabl barsak sendromunun kabızlık ve ishal dönemlerinde değişmektedir. Postprandial semptomatoloji de ishal döneminde daha belirgin olmaktadır. Bu sonuçlar, ishal ve kabızlık sırasında seratoninin plazma düzeylerindeki bu değişiklikler alterne tip irritabl barsak sendromunun patogenezinde yer alabildiğini düşündürmektedir. Ayrıca serum seviyesindeki seratonin değişmeleri semptomatolojinin bazı yönlerini de açıklayabilir.*

Anahtar kelimeler: Alterne tip irritabl barsak sendromu, 5-hidroksitriptamin, şişkinlik, ağrı, aciliyet hissi

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INTRODUCTION

Diagnosis of irritable bowel syndrome (IBS) depends on the evaluation of symptoms and applying the symptoms criteria that have been developed for IBS. The clinicians classify IBS as diarrhea-dominant (D-IBS), constipation-dominant (C-IBS) and alternating-type IBS (A-IBS) according to the primary alteration of bowel function (1,2). Although many factors have been suggested in the IBS etiopathogenesis, it has been very well known that 5-HT has a key function in bowel motility and plays a significant role in the regulation of intestinal and pancreatic secretions (3). Therapeutic successes with serotonin (5-hydroxytryptamine [5-HT])-modulating agents encouraged re-examination of the role of 5-HT in IBS.

The majority of 5-HT (95%) is deposited in enterochromaffin (EC) cells, and a minor amount of 5-HT is found in enteric neurons (5%) in the gastrointestinal (GI) tract, which involves 80% of the body 5-HT. The EC cells act as a transducer by secreting 5-HT after a luminal stimulus such as meal ingestion, pressure and toxins. 5-HT acts on mucosal neurons at the lamina propria and activates secretomotor responses. The information about the condition of the intestine is transmitted to the central nervous system by the visceral afferent pathways. Almost all plasma 5-HT originates from the GI tract (4). After passing into plasma from the intestine, 5-HT is taken up rapidly into platelets or is metabolized to 5-hydroxyindole acetic acid in the liver or kidney (5). The majority of 5-HT in peripheral blood is derived from platelets; however, they cannot make 5-HT (6). Thus, 5-HT concentration measured in platelet-poor plasma reflects transient alteration of 5-HT concentration more accurately.

A standard carbohydrate-rich meal is a good stimulus that increases the platelet-poor plasma 5-HT concentrations in healthy subjects and IBS patients (7). A pilot study by Bearcroft et al. (8) showed that postprandial platelet-poor plasma 5-HT levels were higher in D-IBS female patients than in healthy control subjects. Furthermore, in another recent study, postprandial symptoms were found to be correlated with increased platelet-poor plasma 5-HT concentrations in D-IBS female patients (9). Dunlop et al. (10) reported that C-IBS patients had impaired postprandial 5-HT secretion, and D-IBS patients had higher peak postprandial 5-HT levels. Although many human and animal studies have investigated postprandial platelet-poor plasma 5-HT concentration and its relationship with postprandial symptomatology in D-IBS or C-IBS, there has been no human study about differences in postprandial 5-HT concentrations in A-IBS.

The aim of this study was to evaluate the differences in postprandial platelet-poor plasma 5-HT concentrations during diarrhea and constipation periods of A-IBS patients and to investigate the relationship between platelet-poor plasma 5-HT concentrations and postprandial symptomatology during both periods.

MATERIALS AND METHODS

Patients and Controls

Nine A-IBS patients (female/male: 7/2), diagnosed according to Rome II criteria, and 9 (female/male: 7/2) healthy control subjects were studied. Patients were recruited from the gastroenterohepatology outpatient clinic. Controls were recruited from hospital staff and friends. The inclusion criteria of the study for the all subjects were absence of coexistent diseases (including severe psychiatric and neurological disorders) and of drug abuse (heroin, cocaine, etc), normal hemogram, biochemistry, serologic, stool examination, urinalysis, and colonoscopic findings, systolic blood pressure under 160 mmHg, and diastolic blood pressure under 90 mmHg (11-15). History of GI surgery (excluding appendectomy, cholecystectomy, hiatus hernia operation) or hysterectomy, exacerbated or induced GI symptoms by milk or milky products, taking drugs that modify GI function or the 5-HT system (analgesics, tranquilizers or antidepressants), pregnancy, breast-feeding, menopause, alarm symptoms, age under 18 years or over 65 years, alcohol consumption of more than 40 g/week and smoking more than 5 cigarettes per day constituted the other exclusion criteria.

All the patients and control subjects gave informed consent for their participation in the study and the local ethics committee approved the protocol. The study was performed according to the Declaration of Helsinki.

Study Protocol

Routine biochemical tests, hemogram and urinalysis were performed in all the studied subjects. The patients were investigated for the presence of anti-endomysium IgA, and stool analysis was made during diarrhea and constipation periods to exclude the presence of underlying malabsorption

syndromes or infectious pathologies. Serotonin-rich foods such as walnut, banana, aubergine, tomato, hazelnut, peanut, and avocado were stopped 72 hours (h) before the study. All the drugs and cigarette smoking were forbidden prior to 48 h before the study, and alcohol and caffeine were ceased 24 h prior to the study. Female patients and female controls were investigated during the luteal phase of the menstrual cycle.

Constipation and diarrhea periods were determined according to stool frequency and/or consistency. Defecation less than 3 times per week or hard, lumpy stool was defined as "constipation", whereas defecation more than 3 times/day or soft, watery stool was defined as "diarrhea" (15). Patients were investigated for the postprandial platelet-poor plasma 5-HT concentrations while they had diarrhea or constipation in at least the last 72 h. Patients and controls presented to the laboratory at 8:30 a.m. after an overnight fast. Patients presented for the investigation twice, during diarrhea and during constipation, while controls presented only once. An arm vein was cannulated and 10 ml blood was taken for platelet count and 5-HT analysis. One hour later, a carbohydrate-rich 475 kcal breakfast (consisting of 50 g cheese, 3 spoons of black cherry jam, 20 g butter, and 50 g wheat bread) was eaten within 10 minutes (min). Over the following 3 h, 10 ml venous blood samples were taken at half-hour intervals. At the end of the procedure, the cannula was withdrawn.

Symptomatology evaluation was initiated with a telephone call before the patient presented to the laboratory inquiring whether or not his/her IBS was active. If active, the patient was queried regarding the current period of the disease. In addition, after presentation to the laboratory, once while fasting and 6 times postprandially at half-hour intervals, patients were asked questions targeting the presence and severity of abdominal pain/discomfort, bloating and bowel urgency, such as, "In the past half hour have you experienced abdominal pain/discomfort?". If the subjects reported "yes", they were then asked to grade the severity of that symptom using the scale 1=mild, 2=moderate, 3=intense, or 4=severe. A worsening of symptoms with meal ingestion was defined as an increase in symptom score of at least 1. "5=defecation" was used only for bowel urgency.

Analysis of Platelet-Poor Plasma 5-HT Concentration

Blood samples were taken into the tubes containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at 5000 rpm for 10 min. Supernatant was aspirated to a second tube containing EDTA and further centrifuged at 5000 rpm for 10 min to obtain a platelet-poor plasma. To ensure that platelet-poor plasma was obtained, platelet count was measured in the supernatant of the twice-centrifuged blood samples. A further 2 ml of platelet-poor plasma supernatant was aspirated and stored in Eppendorfs at -70°C for later 5-HT analysis. The samples were later transported to the laboratory with maintenance of the cold chain for batch analysis. Laboratory assistants were blinded to the subject status.

Plasma 5-HT concentrations were measured by high-performance liquid chromatography (HPLC). The frozen plasma samples were opened at room temperature in the laboratory. Plasma (100 µl) and 200 µl Chromsystems kit were mixed and then left for 10 min after mixing. The mixture was centrifuged and supernatant was submitted to HPLC (HP Agilent 1100, Germany). The reading was done by electrochemical detector (ECD detector, Germany) in 6-10 min. The sensitivity of the HPLC method has been reported in several previous studies (9). The sensitivity of the kit for detection of 5-HT was at least 3 ng/ml, but 5-HT levels <3 ng/ml could be measured with use of HPLC. The recovery of the test was 95%, intra-assay coefficient of variation (CV) was 1.9-2% and inter-assay CV was 2.6-2.7%.

No medication was used during the study. None of the symptoms and signs caused by an overnight fasting, such as hypoglycemia and hypoglycemic symptoms, was observed in any patient or control. During the 4-h observation, 70 ml of blood was taken from control subjects. The procedure was performed twice in patients; thus, 140 ml of blood was taken from patients. This amount of blood withdrawal did not cause any abnormalities such as hypotension or weakness.

Data and Statistical Analysis

The controls were assigned as "condition 1", diarrhea period of patients was assigned as "condition 2", and constipation period of patients was assigned as "condition 3". Fasting and postprandial platelet-poor plasma 5-HT concentrations, symptom scores and differences in 5-HT concentrations and symptom scores postprandially according to baseline, peak 5-HT concentrations and time to reach peak 5-HT concentrations were compared between

the 3 conditions. The scores of fasting and postprandial abdominal pain, bloating and urgency were compared separately and total abdominal pain, bloating and urgency scores at each time point were compared as well.

Differences were considered significant when p-values were <0.05. The Kruskal-Wallis with Dunn's test, a post–hoc test, was used for comparisons between the 3 conditions.

RESULTS

Demographic Characteristics of Patients and Controls

Ages, weights and heights of patients and controls were found to be similar. Median ages of the control and patients groups were 33 years (range: 18-43 years) and 18-46 years, respectively. Job distribution among patients was: student (3), clerk (3), teacher (1) and hospital staff (2) and among controls was: student (1), hospital staff (5), housewife (1), and worker (2).

Duration of Symptoms and Signs and Diagnosis

The median duration of patients' symptoms was 6 years (range: 2-10 years), whereas median duration of diagnosis by a doctor was 1 year (range: 1–9 years).

Constipation periods lasted a median 4 days (range: 3-15 days). All patients complained of hard, lumpy stool passage, straining during defecation and abdominal distension during constipation periods. The change in number of defecations per day was not always associated with change in consistency of stool. All of the patients defecated <3 times per week. Two patients reported mucous passage during constipation.

Diarrhea periods lasted a median 3 days (range: 3-7 days). All patients complained of soft, watery stool passage, abdominal distension and urgency during diarrhea periods. All patients complained of increased number of defecations per day (median: 4 times/day; range: 3-6 times/day). Four patients reported mucous passage during diarrhea.

Platelet-Poor Plasma 5-HT Concentrations

Comparisons of fasting and postprandial plasma 5-HT concentrations of the 3 conditions

Fasting plasma 5-HT concentrations of the 3 conditions were similar (p=0.3). When postprandial values were compared between the 3 conditions, only 5-HT concentration at the 30th min (postprandial 1=PP1) was found to be different (p=0.02). Platelet-poor plasma 5-HT concentrations at PP1 during the diarrhea periods were significantly higher than during the constipation periods (p<0.05). There was no significant difference in 5-HT concentration at PP1 between controls and diarrhea periods or between controls and constipation periods of patients (p>0.05) (Figure 1, Table 1).

Comparisons of differences in 5-HT concentrations postprandially according to baseline between the 3 conditions

The differences in serum 5-HT concentrations postprandially according to baseline were compared between the 3 conditions. The increase in serum 5-HT concentrations at PP1 according to baseline was found to be statistically significant (p=0.002). The increase in serum 5-HT during the diarrhea periods of patients was significantly higher than during the constipation periods of patients and when compared to healthy subjects (p<0.05) (Figure 2, Table 2). However, differences at PP1 according to baseline were not statistically different between healthy subjects and patients during the constipation period (p>0.05).

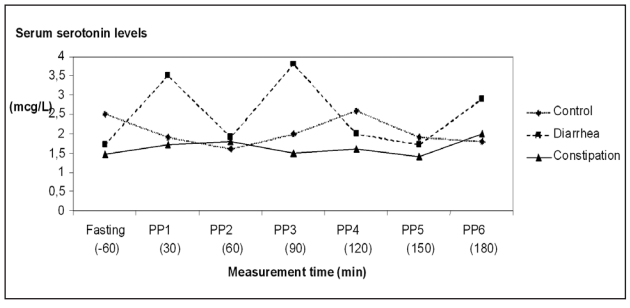

Figure 1. Comparisons of fasting and postprandial plasma 5-HT concentrations of the 3 conditions. PP: Postprandial.

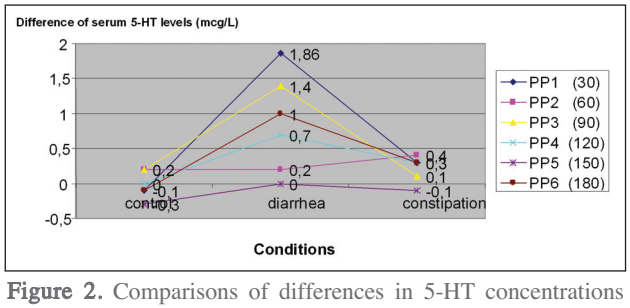

Figure 2. Comparisons of differences in 5-HT concentrations postprandially according to baseline between the 3 conditions. PP: Postprandial.

Table 1. Comparisons of fasting and postprandial plasma 5-HT concentrations of the 3 conditions

		Serum 5-HT level (µg/L)				
	Condition	**Median**	**Minimum**	**Maximum**	X^2_{KW}	**P value**
Fasting	Control	2.5	0.9	4.9	2.389	0.30
	Diarrhea	1.7	0.74	3.05		
	Constipation	1.45	0.9	2.8		
PP1	Control	1.9	0.67	5.1	**7.656**	**0.02**
	Diarrhea	3.5	2.1	24.5		
	Constipation	1.7	0.9	3.7		
PP2	Control	1.6	0.53	5.7	0.535	0.77
	Diarrhea	1.9	1.1	12.4		
	Constipation	1.8	1	3.5		
PP3	Control	2	0.9	18.4	3.853	0.14
	Diarrhea	3.8	1.3	15.5		
	Constipation	1.5	1	3.2		
PP4	Control	2.6	1	22.5	1.563	0.46
	Diarrhea	2	1.3	4.6		
	Constipation	1.6	1	3		
PP5	Control	1.9	1.1	13.8	2.498	0.28
	Diarrhea	1.7	1.1	3		
	Constipation	1.4	0.8	2.9		
PP6	Control	1.8	1.1	3.9	2.59	0.32
	Diarrhea	2.9	1.3	9.2		
	Constipation	2	1	4.4		

X^2_{KW}: Kruskal-Wallis chi-square. PP: Postprandial.

Table 2. Comparisons of differences in 5-HT concentrations postprandially according to baseline between the 3 conditions

		Difference in postprandial serum 5-HT level according to baseline (µg/L)				
	Condition	**Median**	**Minimum**	**Maximum**	X^2_{KW}	**P value**
Fasting	Control	-0.1	-1.63	1.8	12.959	**0.002**
	Diarrhea	1.86	0.9	21.45		
	Constipation	0.3	-0.5	2.4		
PP1 - fasting	Control	0.2	-2.8	2.4	1.236	0.539
	Diarrhea	0.2	-1.68	11.66		
	Constipation	0.4	-1.3	2.05		
PP2 - fasting	Control	0.2	-2.8	15.1	4.418	0.110
	Diarrhea	1.4	-0.2	13.49		
	Constipation	0.1	-1.4	1.75		
PP3 - fasting	Control	0	-2.3	19.2	1.169	0.557
	Diarrhea	0.7	-0.5	2.7		
	Constipation	0.3	-1.8	1.8		
PP4 - fasting	Control	-0.3	-0.9	10.5	1.008	0.604
	Diarrhea	0	-0.55	1.56		
	Constipation	-0.1	-0.8	1.6		
PP5 -fasting	Control	-0.3	-0.9	10.5	1.008	0.604
	Diarrhea	0	-0.55	1.56		
	Constipation	-0.1	-0.8	1.6		
PP6 -fasting	Control	-0.1	-3.1	0.7	5.166	0.076
	Diarrhea	1	-0.95	7.4		
	Constipation	0.3	-0.8	2.8		

X^2_{KW}: Kruskal-Wallis chi-square. PP: Postprandial.

Comparisons of peak 5-HT concentrations and time to reach peak 5-HT concentrations between the 3 conditions

Peak serum 5-HT concentrations were significantly higher during the diarrhea period than during the constipation period (p=0.035). However, peak levels were not statistically different between controls and patients during constipation, or between controls and patients during diarrhea (Figure 3). Time to reach peak 5-HT concentration was similar between the 3 conditions (p>0.05).

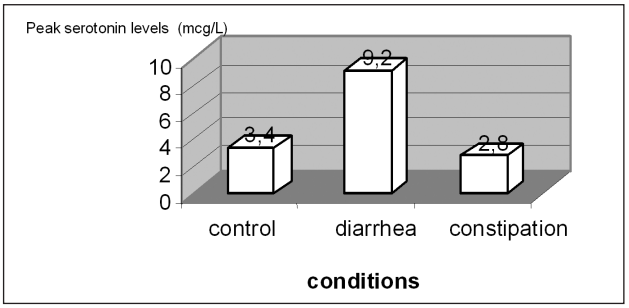

Figure 3. Comparisons of peak 5-HT concentrations between the 3 conditions.

Symptomatology

Comparisons of total symptom scores of the 3 conditions

Fasting total symptom scores between the 3 conditions were found to be different (p=0.009). Fasting PP1 and PP2 total symptom scores during diarrhea and constipation were higher than in controls (p<0.05), whereas diarrhea and constipation periods did not differ (p>0.05) (Figure 4). Total symptom scores at PP4, PP5 and PP6 during diarrhea periods of patients were higher than those of control subjects (p<0.05). There was no significant difference between diarrhea and constipation periods and between constipation period and controls. Total symptom scores at PP3 were similar between the 3 conditions (p>0.05).

Comparisons of differences in postprandial total symptom scores according to baseline

Increase in total symptom scores at PP1 according to baseline during diarrhea was found to be higher than that of the control group (p<0.05). There was no correlation between total symptom scores and serotonin levels.

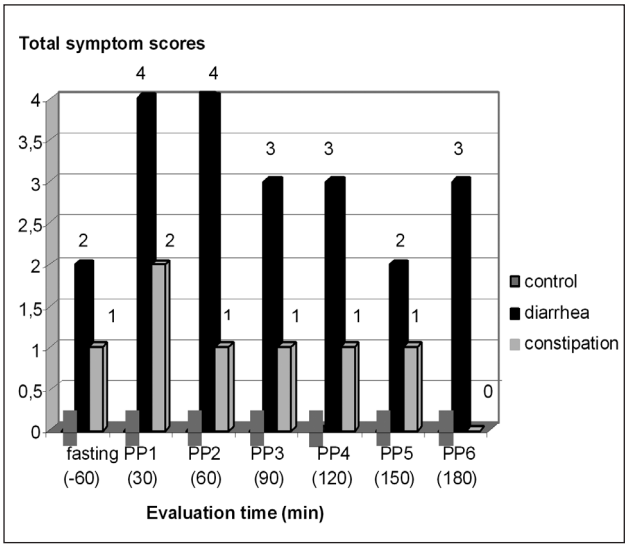

Figure 4. Comparisons of total symptom scores of the 3 conditions. PP: Postprandial.

Comparisons of independent symptom scores in the 3 conditions

There was no significant difference in any type of fasting symptom score between the 3 conditions. The pain, bloating and urgency scores of controls were lower postprandially than in patients during diarrhea (p<0.05). The differences in symptom scores according to baseline were not statistically significant except for PP1 bloating score.

DISCUSSION

Many studies have been carried out recently to explain the pathophysiology of IBS. C-IBS and/or D-IBS patients, post-infective (PI)-IBS patients and healthy subjects were enrolled in these studies. No findings of a human study about A-IBS, indicating those individuals who suffer from both diarrhea and constipation during different time periods, have been reported to date. An animal study with rats was reported (16). To the best of our knowledge, this is the first study to show that platelet-poor plasma 5-HT concentrations after meal ingestion differ during constipation and diarrhea periods in A-IBS patients.

A carbohydrate-rich meal is well known as a good stimulus that increases platelet-poor plasma 5-HT levels in healthy subjects and D-IBS patients (7). In our study, response to meal stimulus was present in healthy subjects but it did not reach statistical significance. Plasma 5-HT levels after meal ingestion during diarrhea periods in patients were found to be higher than during constipation periods, especially 30 min after the meal (p<0.05). However, the platelet-poor plasma 5-HT response to meal was not evident during constipation. Postprandial plasma concentrations of 5-HT were found to be similar or slightly elevated compared with fasting plasma concentrations during constipation. The increase in postprandial plasma 5-HT levels according to baseline was higher during diarrhea than during constipation and when compared to the control group. In the study by Atkinson et al.(17), this increase was not found statistically significant in D-IBS.

Following meal ingestion, platelet-poor plasma 5-HT level begins to rise and remains high for a few hours. However, in some patients' plasma, this gradual increase and decrease is not seen. Sharp

increases and decreases of 5-HT levels were common in previous studies, like in our study (17).

The question "Why does meal ingestion result in increased plasma 5-HT levels sometimes but not at other times in the same patient?" was not answered in this study. The relationship between secretion of 5-HT from the GI tract and platelet-poor plasma 5-HT levels can be affected by many variables, such as serotonin reuptake transporter (SERT) expression, activity, thrombocyte fragility, reuptake of serotonin into bowel, and transport to the liver, lung and platelets. The continuous secretion of serotonin in high concentrations necessitates the evolution of effective mechanisms for its inactivation. The receptors whose ligands are 5-HT desensitize like all other receptors over the course of time. The enzyme-mediated catabolism of signal molecules is a well-known mechanism in transmitter inactivation. Serotonin is inactivated by monoamine oxidases and transferases or other enzymes in the gut. All these enzymes are intracellular molecules. Serotonin inactivation occurs mainly by uptake of serotonin into cells that secrete serotonin and into adjacent cells via SERT molecules (18,19). Previous studies have shown that changes in SERT functions resulted in various alterations in intestinal motility. Selective serotonin reuptake inhibitors (SSRI) may increase orocecal transit but may cause constipation by inhibition of peristalsis as well (20,21). Although these differences in bowel habits were explained by different doses of SSRI, it was striking that D-IBS occurred in some SERT null mouses and C-IBS developed in other SERT null mouses. Only one SERT null mouse had A-IBS (16). SERT -/- mice may develop watery diarrhea as a result of 5-HT-mediated secretory reflex. Transient constipation may be induced by 5-HT receptor desensitization. The various responses to hindered serotonin reuptake may be explained by alteration in the balance between the receptor activation and desensitization.

It has been reported that different 5-HT levels during constipation and diarrhea may be consistent with motor abnormalities in IBS patients (22-24). However, whether alteration of platelet-poor plasma 5-HT levels is a result or a cause of these motor abnormalities could not be explained by our study. The similar time to peak serum 5-HT levels in the 3 conditions showed that postprandial 5-HT concentrations could be related to something other than intestinal transit. Moderate GI transit abnormalities in IBS patients have been reported (25,26). Thus, it is difficult to deduce that change in transit is the only factor that induces increased postprandial plasma 5-HT concentration during diarrhea and causes inadequate increase during constipation.

The control subjects reported no complaint during fasting or postprandially, whereas patients expressed more symptoms both during diarrhea and constipation periods. The symptoms during diarrhea were more severe than those of controls; however, there was no significant difference in severity between constipation and controls, and between constipation and diarrhea. Unlike Houghton et al. (9) reported, there was no correlation between severity of symptoms and serum 5-HT levels. This indicates that mechanisms other than 5-HT concentrations play a role in the severity of the symptoms. Another study indicating that 5-HT is correlated with bowel motility rather than abdominal pain or bloating has been performed with SSRI (27). Administration of SSRI resulted in increased diarrhea incidence, but did not improve symptoms. Increased neuroendocrine response and altered visceral perception during or following stress in IBS patients may explain some of the stress-related GI symptoms (28). Presentation to the hospital for examination or at the onset of altered bowel function may induce stress and may explain why they tend to report many more symptoms during constipation, although plasma 5-HT levels do not increase.

Most human peripheral blood 5-HT originates from the GI tract. Platelets cannot produce 5-HT; however, they quickly uptake circulating 5-HT via SERT. Thus, platelet-poor plasma 5-HT levels were thought to reflect the amount of recently synthesized and secreted 5-HT in EC cells. Many studies have shown that when the blood taken into EDTA-containing tubes was centrifuged at high speed, there was little or no 5-HT secretion into the plasma from platelets (8,29). In our study, the platelet concentration in centrifuged plasma was under 10,000/µl in all studied subjects, so all centrifuged plasma was accepted as platelet-poor plasma.

Because of present evidences that hormones of the steroid ovarian axis may affect the 5-HT system, the female subjects among both patients and controls were investigated during the luteal phases of their menstrual cycles. Estrogen and progesterone

concentrations are in balance during the luteal phase (30).

The major limitations of our study are the limited number of subjects and the absence of analysis of 5-HT in tissue specimens, metabolites in serum and tissue specimens and SERT activity.

In conclusion, the possible role of 5-HT in A-IBS pathophysiology was investigated in this study. We showed that plasma serotonin levels increased postprandially during diarrhea, whereas during constipation, 5-HT response to meal ingestion was absent or inadequate in the same individuals. More extended studies that measure mucosal 5-HT concentrations, amount of EC cells, SERT activity, and 5-HIAA during constipation and diarrhea periods in A-IBS patients should be performed to explain the importance of 5-HT in A-IBS symptomatology and pathophysiology.

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