Introduction

On November 26 and 27 in 2010, the first Asian Pacific Topic Conference was held in Tokyo as a joint meeting organized by the Japanese Society of Gastroenterology (JSGE) and Asian Pacific Association of Gastroenterology (APAGE). As emerging common disorders in the Asia- Pacific region, functional gastrointestinal disorders (FGIDs) was chosen as the topic, and more than 40 researchers in this field from different Asia-Pacific region participated in the meeting. Information on experiences of participants was collected by questionnaires.

FGIDs are considered to be important to public health because they are highly prevalent, induce major social and economic burdens, and are associated with impaired health-related quality of life. Since FGIDs are a heterogenous group of chronic conditions, they have different clinical features and probably have different underlying pathophysiologic mechanisms.[1] Although there are established diagnostic criteria such as Rome III,[2–4] the boundary between true abnormality and health remains to be defined, more effective therapy should be challenged on many levels, and establishing effective clinical guidelines specific for the Asia-Pacific (A-P) region is necessary.

It is also important to know how diagnosis and management have been conducted in different regions of Asia because clinical approach to FGID could be largely affected by heterogeneity in disease structure and socioeconomic conditions in each country. We therefore collected data by questionnaires in order to determine the most common clinical approach in diagnosis and treatment (management) of FGID in the A-P region. The information we obtained may be useful for understanding the current situation of diagnosis and treatment of FGIDs in the A-P area.

Methods

In September 2010, questionnaires were sent to 43 physicians and researchers in the field of FGIDs in the A-P region who were scheduled to attend the first Asian Pacific Topic Conference in Tokyo. Twenty faculties and investigators from Asian Pacific Societies of APAGE and 23 faculties from Japan were invited to take part in the questionnaire survey. On October 30, a reminder was sent to those who had not responded.

The questionnaire consisted of 60 multiple-choice questions concerning physician's preference about diagnosis and management of functional dyspepsia (FD) and irritable bowel syndrome (IBS). The questionnaire included 29 items for FD, and 31 items for IBS. A comment could be added next to some questions.

In the questionnaire we excluded questions about definition, pathophysiology, etiology and epidemiology of FGID. Diagnosis section included the following questions: (i) How is FGID diagnosed by a general practitioner (primary care physician) or by a gastroenterologists (GI specialists)? (ii) How are organic diseases or other diseases excluded at diagnosis? (iii) How are FGID patients categorized into subgroups? (iv) How are characteristic symptoms, life styles, and dietary conditions taken into account? and (v) How are psychological and physiological examinations performed at diagnosis? The treatment section included the following questions: (i) How is the disease explained and what advice is given about life style and diet? (ii) When and what kinds of GI drugs are preferentially administered to patients? and (iii) When and what kinds of psychological drugs and psychological therapy are used for patients?

Statistical data were analyzed using the chi-square test, and a P-value of < 0.05 was considered statistically significant.

Results

Diagnosis of FD and IBS (general practitioners *vs* gastroenterologists)

There was a similarity in clinical approaches of the two disorders except for some items. For example, as shown in Figure 1, the term "FD" was used by only 40% of general practitioners (primary care

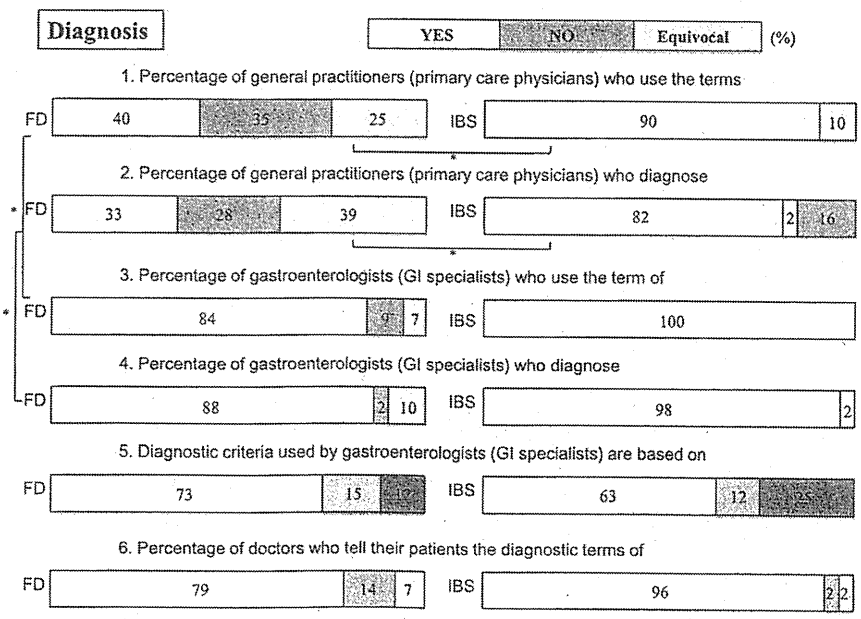

Figure 1 Diagnosis of functional dyspepsia (FD) and irritable bowel syndrome (IBS) (general practitioner *vs* gastroenterologist) in the Asia-Pacific region. Results in answer to items No.1–6 are shown as percentages of doctors except No. 5. No. 5: ▭ Rome III, ▭ Rome II, ▭ others. The left side of the figure shows answers for FD, and the right side shows the answers for IBS. The overall answers from 43 panelist doctors are shown. An open bar indicates the percentage of "yes" answers, a dark-shaded bar indicates the percentage of "no" answers, and a light-shaded bar indicates the percentage of "equivocal" answers. * $P < 0.05$.

physicians) in the A-P region, and only 33% of general practitioners diagnosed FD. Especially in Japan many cases were diagnosed as other disorders such as chronic gastritis. On the other hand, the term "IBS" was used by 90% of general practitioners and 82% of general practitioners diagnosed IBS ($P < 0.05$).

The terms FD and IBS were used by 84% and 100% of gastroenterologist (GI specialists), respectively, and these disorders were diagnosed by 88% and 98% of gastroenterologists, respectively. Diagnosis of FD or IBS by gastroenterologists was based mainly on Rome III criteria (73% for FD and 63% for IBS) and sometimes on Rome II criteria (15% for FD and 12% for IBS). Diagnosis in other cases was usually based on clinical symptoms, criteria that are broader than the Rome criteria, excluding organic diseases. There was no significant difference between Japan and other area in the A-P region in terms of use of the diagnostic criteria. After diagnosis of FD or IBS, most of the panelists told patients the diagnostic term FD (79%) or IBS (96%).

Investigations and alarm signs of FD and IBS

As shown in Figure 2 and 68% of the panelist doctors mandatorily performed upper GI endoscopy for diagnosis of FD. The performance rate was significantly greater among Japanese doctors than other A-P doctors. For those who did not mandatorily perform upper GI endoscopy, the main indications for endoscopy were age (over 50 years) (64%), presence of alarm features (64%), and use of NSAIDs (non-steroidal anti-inflammatory drugs) (57%). The dominant features that panelists regarded as alarm signs in patients with dyspepsia were weight loss (91%), anemia or GI bleeding (86%), age (over 50 years)(81%), family history of gastric cancer (77%), dysphagia (77%), past history of ulcer (72%), and vomiting (67%).

Less than half of the doctors (45%) usually performed imaging studies of the lower GI tract for the diagnosis of IBS. For those who did not perform lower GI examinations, the main indications for colonoscopy were the presence of alarm features (68%), positive fecal occult blood (65%), age (over 45 years) (55%), and family history of colon cancer (55%). The dominant features that panelists regarded as alarm signs in patients with IBS symptoms were blood in stools (98%), presence of anemia (93%), weight loss (86%), family history of colon cancer (76%), age over 45 years (74%), and signs of inflammation (74%).

When panelists diagnosed FD, 75% of the doctors usually performed blood examinations to exclude organic diseases. Interestingly almost all of the Japanese doctors performed blood examinations, while only half of other A-P doctors did ($P < 0.05$).

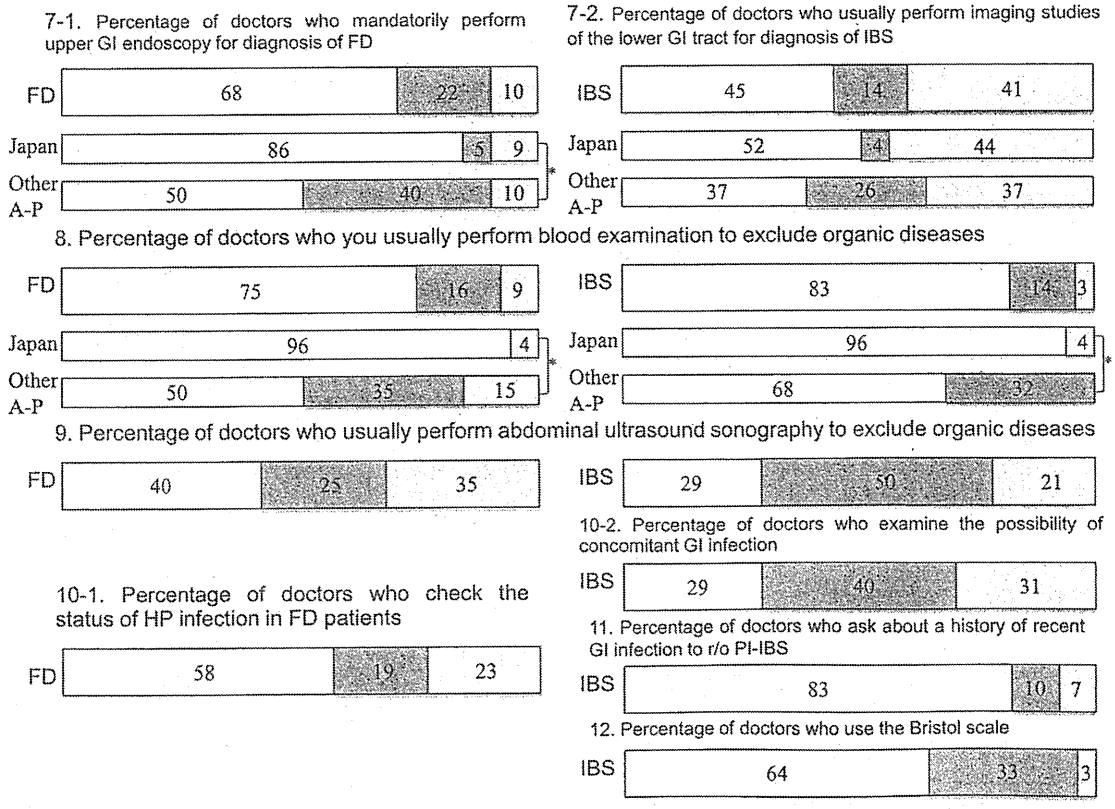

Figure 2 Preferences of panelist doctors in the Asia-Pacific region in diagnosis of FD and IBS (about differential diagnosis). Results in answer to items No.7–12 are shown as percentages of doctors. The overall answers from 43 panelist doctors are shown, except for Japan with answers from 23 doctors, and Other A-P, with answers from 20 doctors. An open bar indicates the percentage of "yes" answers, a dark-shaded bar indicates the percentage of "no" answers, and a light-shaded bar indicates the percentage of "equivocal" answers. A-P, Asia-Pacific region; HP, *Helicobacter pylori*; PI-IBS, Post-infectious IBS. $*P < 0.05$.

The items usually examined were full blood count (86%), AST, ALT and ALP (alkaline phosphatase) (81%), total protein and albumin (65%), CRP (C-reactive protein) or ESR (erythrocyte sedimentation rate) (58%), and others including blood glucose or HbA_1C, thyroid function, renal function and electrolytes, serum amylase especially for Japanese doctors, and serological tests for celiac disease especially for other A-P doctors. In the case of IBS, 83% of doctors usually performed blood examinations of items similar to those for FD, such as full blood count (86%), CRP or ESR (81%), AST, ALT and ALP (79%), and total protein and albumin (64%). Other items examined were thyroid function, renal function, blood glucose or HbA1C, tumor markers such as CEA (carcinoembryonic antigen) especially among Japanese doctors, serological tests for celiac disease, milk intolerance and a test for bacterial overgrowth especially among other A-P doctors. On the other hand, when panelists diagnosed FD or IBS, fewer doctors (40% for FD and 29% for IBS) usually performed abdominal ultrasound sonography to exclude organic diseases.

Differential diagnosis, sub-grouping, and other examinations

It was shown that only 58% of doctors checked the status of *Helicobacter pylori* (HP) infection in FD patients, with no significant difference between Japanese and other A-P doctors. In patients with IBS, only 29% of panelist doctors examined the possibility of concomitant GI infection, although many doctors (83%) asked patients about past history of recent GI infection to rule out post-infectious IBS. Infectious agents considered in IBS patients included *Salmonella, Yersinia, Escherichia coli* and *Mycobacterium* in Japan and *Giardia lamblia*, parasites, and bacterial overgrowth in other A-P region. In the case of FD, other infectious agents than HP were usually not taken into account by panelists.

As shown in Figure 3 when panelists diagnose FD, 54% of doctors subcategorized their patients mostly (87%) by using Rome III diagnostic criteria. There was no difference in sub-grouping between Japanese and other A-P doctors. In the case of IBS, however, subgrouping of patients appeared to be more popular among all panelist doctors (80%) ($P < 0.05$), and most of them (87%) subcategorized their patients into IBS-C, IBS-D or IBS-M according to the Rome III criteria. Bristol stool form scale was used by 64% of doctors when they asked patients about stool pattern.

When panelists diagnosed FD, they usually excluded patients with reflux symptoms (54%), but 29% of the panelists did include these patients. In contrast, when panelists diagnosed FD, they usually included patients with lower GI symptoms suggesting IBS (63%), and only 28% of panelists excluded these patients. For diagnosis of IBS, 71% of doctors took into account other symptoms such as bloating, meal-related abdominal pain, gas, and urgent sensation. About half (52%) of the panelists, especially

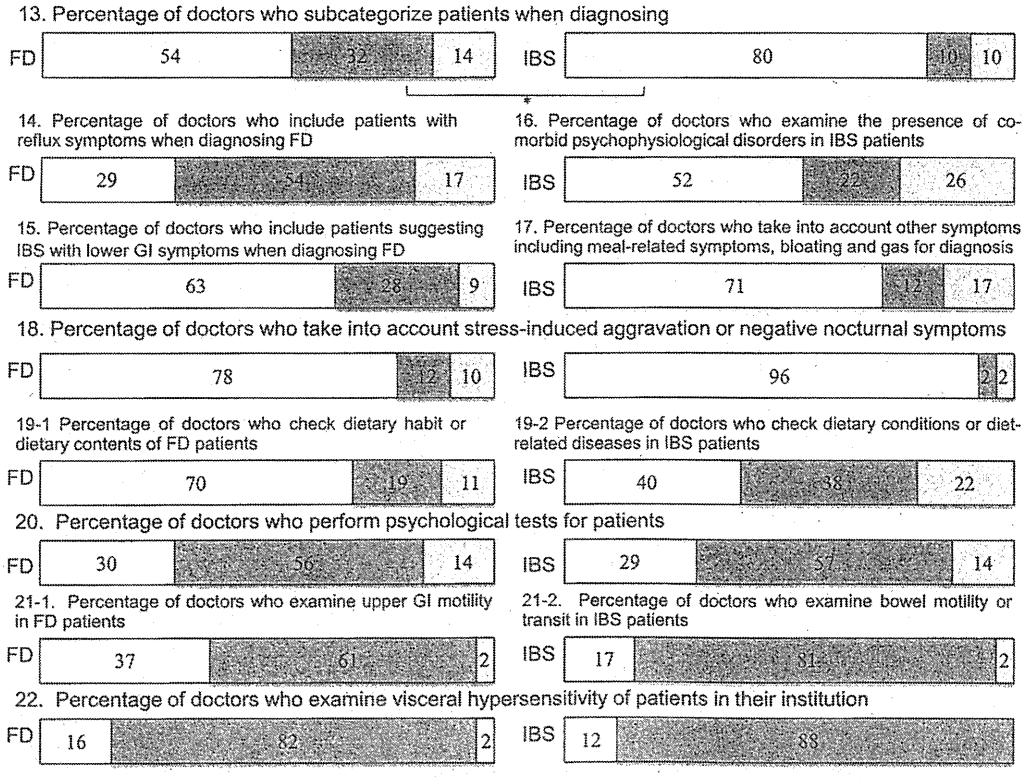

Figure 3 Preferences of panelist doctors in the Asia-Pacific region in diagnosis of FD and IBS (sub-grouping and other examinations). Results in answer to items No.13–22 are shown as percentages of doctors. The overall answers from 43 panelist doctors are shown. An open bar indicates the percentage of "yes" answers, a dark-shaded bar indicates the percentage of "no" answers, and a light-shaded bar indicates the percentage of "equivocal" answers. $*P < 0.05$.

other A-P doctors (74%), examined for the presence of co-morbid psychophysiological disorders in IBS patients.

For diagnosis of FGIDs, most of the panelists took into account stress-induced aggravation or disappearance of nocturnal symptoms, particularly for diagnosis of IBS (96%). Seventy percent of panelist doctors checked the dietary habit or dietary contents in their FD patients, although only 16% of doctors examined food allergies in these patients. Forty percent of panelist doctors checked dietary conditions or diet-related diseases in IBS patients to rule out conditions such as food allergy, or celiac disease. This item tended to be more widely checked among doctors in other A-P regions (53%) than in Japan (30%). Diagnostic tests mainly used for this item were serological tests for celiac disease, hydrogen or lactose breath test, and serum IgE and RAST (radioallergosorbent test).

For specific examinations, about 30% of the panelists performed psychological tests for their FD or IBS patients using various questionnaires concerning depression and anxiety such as HADS (hospital anxiety and depression scale), SDS (self-rating depression scale), PHQ (patient health questionnaire)-9, HAMD (Hamilton rating scale for depression), STAI (state-trait anxiety inventory), and other personality and psychosomatic tests including CMI (Cornell medical index), SCID (structured clinical interview for DSM), MMPI (Minnesota multiphasic personality inventory) and Egogram. Upper GI motility was examined in 37% of FD patients in institutes of panelist doctors by various methods such as ultrasound, ^{13}C-acetate breath test, scintigraphy, marker transit, electrogastrogram (EGG), and manometry. The frequency of bowel movement or bowel transit examination was less (17%) than upper GI, and radio-opaque marker, manometry and breath hydrogen test were mainly used for this examination. Visceral hypersensitivity of FGID patients was examined in only 16% of FD patients by barostat and water drinking test and in only 12% of IBS patients by barostat. However, in some institutions, functional brain activity was also determined by using evoked potential, positron emission tomography (PET), and/or functional magnetic resonance imaging (f-MRI).

Treatment of FGID—Life style and dietary factors

For the treatment of FGID, about 80% of panelist doctors explained the possible pathophysiology of FD or IBS to their patients (Fig. 4), with no difference between Japan and other A-P regions. For treatment of FD, almost all (95%) of the doctors gave advice about improvement of life style (including stress coping) or dietary habit to the patients, and 85% of the doctors taking time for the explanation, and 69% of the panelists thought that the advice given was more important than medication. Similarly, for treatment of IBS, almost all (95%) of the panelist doctors gave advice about improvement of life style (including stress coping), and 79% of the doctors gave advice about improvement of dietary habit or

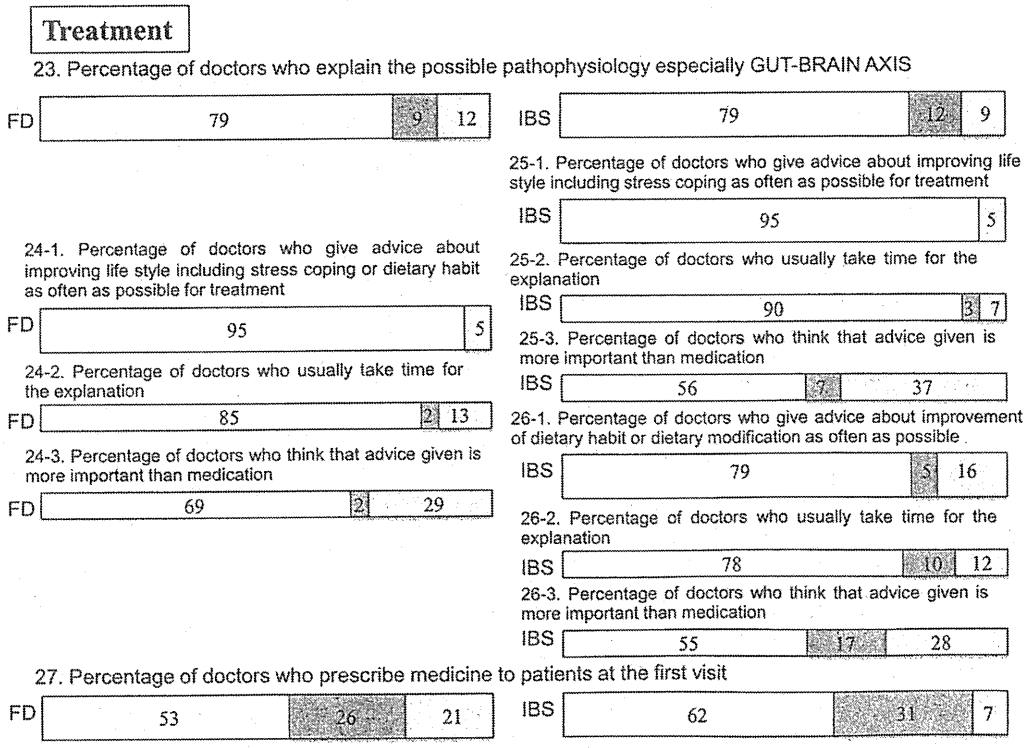

Figure 4 Preferences of panelist doctors in the Asia-Pacific region in management (treatment) of FD and IBS (general aspects). Results in answer to items No.23–27 are shown as percentages of doctors. The overall answers from 43 panelist doctors are shown. An open bar indicates the percentage of "yes" answers, a dark-shaded bar indicates the percentage of "no" answers, and a light-shaded bar indicates the percentage of "equivocal" answers.

dietary modification, usually taking time for the explanation. However, only about half of the panelists thought that advice for life style (56%) or dietary modification (55%) was more important than medication, and less than half of the Japanese doctors thought so (life style, 41%; dietary modification, 48%).

Treatment of FGID—pharmacotherapy

About half of the panelist doctors (53%) usually prescribed medicine to patients complaining of dyspeptic symptoms at the first visit. If not, they prescribed medicine immediately after diagnosis of FD (61%), after communication with patients to keep a good relationship (22%), or according to the patient's request (13%). In the case of IBS, about 2/3 of the panelist doctors (62%) usually prescribed medicine to patients complaining of IBS-like symptoms at the first visit. If not, they prescribed medicine immediately after diagnosis of IBS (54%), after communication with patients to keep a good relationship (23%), or according to the patient's request (15%).

For treatment for subgroups of FD, about 68% of the panelist doctors usually gave different prescriptions to patients with PDS (postpradial distress syndrome: patients with meal-induced dyspeptic symptoms) and patients with EPS (epigastric pain syndrome: patients with epigastric pain) type (Fig. 5). About 3/4 of the doctors prescribed a histamine H2-receptor antagonists (H2RA) to patients with FD, 71% of doctors prescribed H2RA to EPS patients, and 23% of doctors prescribed H2RA to all patients. Most of the panelist doctors (89%) prescribed PPI (proton pump inhibitors) to patients with FD, 49% of doctors prescribed PPI to EPS patients, and 35% of doctors prescribed PPI to all patients. Interestingly, all panelist doctors (100%) prescribed some kind of prokinetics to patients with FD, 66% of doctors prescribed prokinetics to patients with PDS type, and 22% of doctors prescribed ptrokinetics to all patients. Mosapride citrate, domperidone, itopride hydrochloride, and Chinese herbal medicine were frequently used. In some institutes, motilium, levosulpiride, and simethicone were also used.

When HP was positive, about half (53%) of the panelist doctors eradicated HP in their FD patients. Only 35% of the doctors in Japan said yes for HP eradication, and many doctors (39%) answered equivocal, while 75% of other A-P doctors said yes ($P < 0.05$).

Regarding medication for different subgroups of IBS, about half (50%) of the panelist doctors prescribed some common drugs and about 20% prescribed mostly common drugs among IBS subtypes, and only 26% of doctors prescribed totally different drugs between IBS-C and IBS-D. The commonly used drugs among IBS subtypes were probiotics, polycarbophyl calcium, antispasmodic, prokinetics and anti-depressants. Low FODMAP (fermentable oligo-, di- and mono-saccharides, and polyols) diet and fiber supplementation were also recommended in the Oceania regions. Probiotics were widely used (about 3/4) for patients with IBS. Mild laxatives (92%) and anti-diarrheal drugs including loperamide hydrochloride (85%) were also widely used. Polycarbophil calcium was also popular but mainly in Japan (86%). Lubiprostone is not currently available in the A-P area. The use of prokinetics for IBS was 100% as in the case of FD, and anti-spasmodic (76%), trimebutine maleate (60%), 5-HT3 receptor antagonists including ramosetoron (57%), and 5-HT4 agonists including mosapride citrate (48%) were all used, with trimebutine maleate (78%) and the 5-HT3 antagonist ramosetoron hydrochloride (83%) being preferentially used in Japan. Interestingly, traditional herbal medicine was widely used for FD as well as for IBS in Japan and China but not in the other A-P area. Rikkunshito (Liu-Jun-Zi-Tang in Chinese) was very popular for FD, while Daikenchuto (Da-Jian-Zhong-Tang in Chinese) was also very popular for IBS. Other Chinese traditional medicines used were Hangeshashinnto, Simotang for FD, and Keishika-shakuyakuto for IBS.

Treatment of FGID with psychological drugs and psychotherapy

As shown in Fig. 6, for treatment of FGID, most of the panelist doctors prescribed anxiolytic drugs to patients with FD (90%) and to patients with IBS (83%). Most of the doctors prescribed anxiolytic drugs as second-line therapy for FD (72%) and IBS (74%), but others prescribed anxiolytic drugs as third-line therapy. Anxiolytic drugs were prescribed to FGID patients mostly with anxiety signs (70% in FD and 67% in IBS), but about 1/5 of patients were prescribed drugs when they showed anxiety by psychological tests.

A large percentage of panelist doctors also prescribed anti-depressants to patients with FD (84%) and to patients with IBS (76%). Two thirds of the doctors prescribed anti-depressants as second-line therapy for FD (70%) and for IBS (71%), but others prescribed anti-depressants as third-line therapy. Anti-depressants were prescribed to about half of FGID patients mostly with depressive signs (57% in FD and 54% in IBS), but about 1/5 of the patients were prescribed anti-depressants when they were found to have depression by psychological tests. A small percentage of doctors prescribed anti-depressants to all patients (8% in FD and 5% in IBS). When doctors prescribed anti-depressants, most of them prescribed low doses (78% in FD and 83% in IBS), but others prescribed regular doses. The anti-depressants preferentially used were tricyclic anti-depressant (41% in FD and 46% in IBS) and selective serotonin reuptake inhibitors (SSRI) (48% in FD and 39% in IBS). Serotonin & norepinephrine reuptake inhibitors (SNRI) were used for about 10–15% (11% in FD and 15% in IBS) of FGID patients.

Specific psychological treatment besides medication was used for about 24% of FD patients and 26% of IBS patients in the institutions of panelist doctors by several methods including cognitive behavioral therapy (CBT), relaxation, meditation, autogenic training, hypnotherapy, and fasting therapy.

Discussion

In this survey, we were able to compare the clinical approaches to FD and IBS by the panelist doctors. Many aspects of diagnosis and treatment of FD and IBS were quite similar, though there were some differences among APAGE countries. For example, the term FD is less commonly used by general practitioners in the A-P region compared with the term IBS, and general practitioners usually do not diagnose FD, while they often diagnose IBS. On the other hand, it was shown that the terms FD and IBS are both frequently used and that they are equally well diagnosed by gastroenterologist (GI specialists). One reason is that the concept of

FD was established only after the publication of Rome II,[5] whereas the concept of IBS has been used for a longer time. Another reason is that other terms such as "chronic gastritis" have also been used in Japan for a clinical condition corresponding to FD. Similarly, sub-grouping categorization was more popular in IBS than FD, and this is because newly defined entities of PDS and EPS were only recently established in Rome III.[3]

It was interesting that 25% of GI specialists used criteria other than Rome II or III for diagnosis of IBS, including Manning diagnostic criteria or BMW (bowel motility workshop) criteria, the

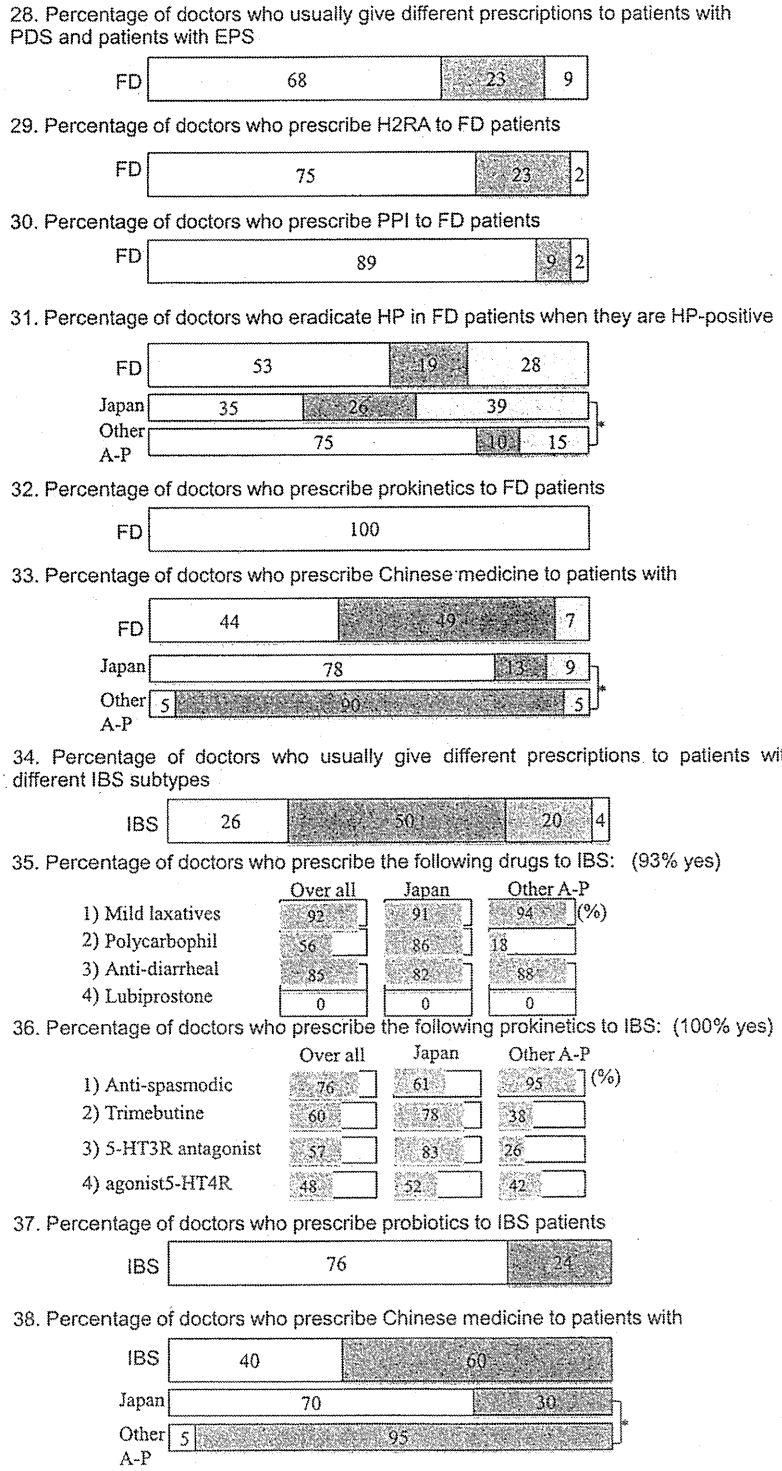

Figure 5 Preferences of panelist doctors in the Asia-Pacific region in management (treatment) of FD and IBS (about medication of GI drugs). Results in answer to items No.28–38 are shown as percentages of doctors. The overall answers from 43 panelist doctors are shown, except for Japan with answers from 23 doctors, and Other A-P, with answers from 20 doctors. An open bar indicates the percentage of "yes" answers, a dark-shaded bar indicates the percentage of "no" answers, and a light-shaded bar indicates the percentage of "equivocal" answers, except No.34-36. No. 34: ▭ yes, ▨ no: partially common, ▭ no: mostly common, ▭ equivocal. In item No.35 and No.36, percentage of prescription of indicated drugs is shown. PDS, postpradial distress syndrome; EPS, epigastric pain syndrome; H2RA, histamine H2-receptor antagonists; PPI, proton pump inhibitors. *$P < 0.05$.

latter especially in Japan.[6] However, there were many common features in approaches for treatment of FD and IBS. The use of prokinetics was very popular, and prokinetics were almost always used for FD as well as for IBS. Prescription of anxiolytic drugs and anti-depressants was also popular for both disorders.

In this survey, only a few differences were found between Japanese and other A-P panelist doctors in diagnosis and treatment of FD and IBS. Most of the doctors (86%) in Japan mandatorily performed upper GI endoscopy for diagnosis of FD and frequently performed blood examination to rule out organic diseases, but only half of other A-P doctors did these examinations. This may be due to the difference in public insurance systems, but another reason may be the greater general concern about gastric cancer in Japan than in other A-P countries. However, it is also interesting that abdominal ultrasound sonography was not usually performed to exclude organic diseases in the diagnosis of FD and IBS even in Japan.

The overall percentage of examination for HP infection status was 58% with no significant difference between Japanese and other A-P doctors. However, surprisingly, for the frequency of HP-eradicative therapy in HP-positive FD patients was significantly lower (35%) in Japan than other A-P regions (75%). This may be because public insurance in Japan does not cover eradication of HP in patients with FD or gastritis. However, this situation may change because the Japanese Society for Helicobacter Research has recently published guidelines recommending eradication for all HP-infected patients.[7] Taking into account that the incidence of gastric cancer is higher in Japan and Korea than in other population area,[8] HP eradication should be more widely considered for HP-positive patients with investigated dyspepsia as recently recommended by the Asia Pacific Consensus on HP infection.[9]

Although not statistically significant, other A-P doctors more frequently checked the dietary conditions or diet-related diseases than did Japanese doctors, especially for IBS patients, to rule out, for example, food allergy, or celiac disease.[10] For the diagnosis of FGID, serological tests for celiac disease were rarely performed. Although celiac disease appears to be extremely rare in Japan,[11] the incidence may increase in the future due to globalization. In other countries, particularly India, Australia and New Zealand, the prevalence of celiac disease is high. For IBS, most of the doctors asked about a history of recent infection in consideration of post-infectious IBS, but they did not usually (only 30%) check concomitant GI infection or bacterial overgrowth. For FD, although almost none of the doctors checked for infections other than HP, the potential relationship between dys-

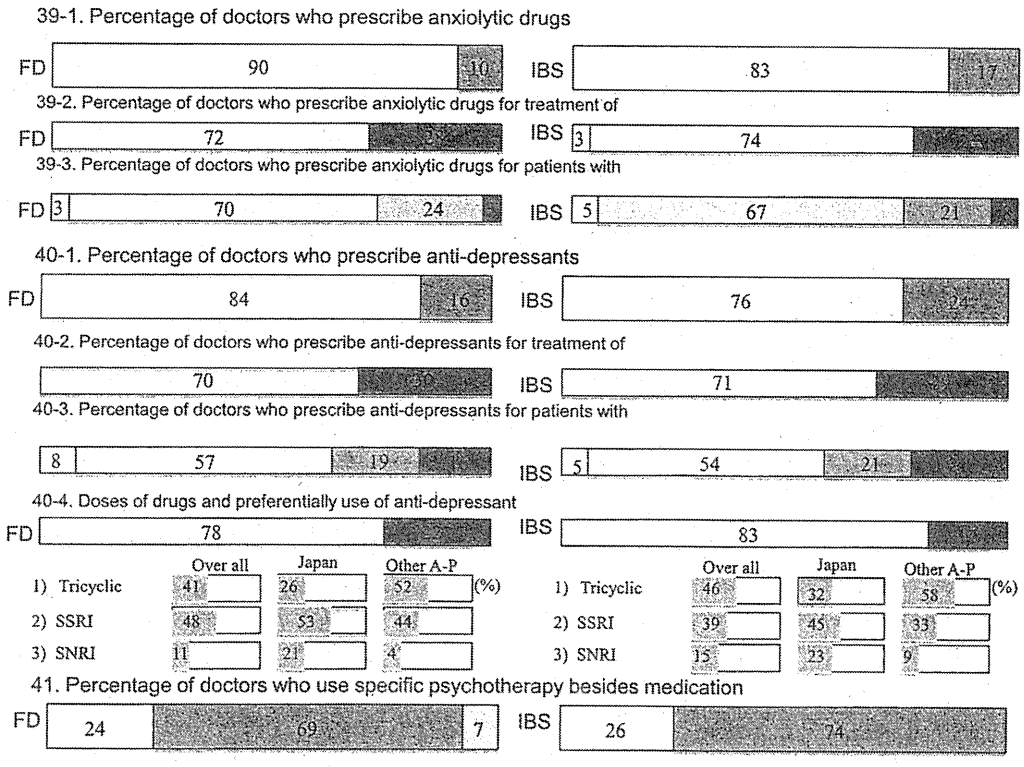

Figure 6 Preferences of panelist doctors in the Asia-Pacific region in management (treatment) of FD and IBS (about medication with psychological drugs and psychological therapy). Results in answer to items No.39–41 are shown as percentages of doctors. The overall answers from 43 panelist doctors are shown. An open bar indicates the percentage of "yes" answers, and a dark-shaded bar indicates the percentage of "no" answers, except some items. No. 39-2, and 40-2: ▭ first line, ▭ second line, ▭ others (third line). No. 39-3, and 40-3: ▭ all, ▭ with anxiety or depressive signs, ▭ positive test, ▭ others. No. 40-4: ▭ low dose, ▭ regular dose. In the lower part of item No.40-4, percentage of prescription of indicated drugs is shown. Tricyclic, tricyclic anti-depressant; SSRI, selective serotonin reuptake inhibitors; SNRI, serotonin & norepinephrine reuptake inhibitors. $^*P < 0.05$.

peptic symptoms and acute gastroenteritis should be investigated further because the pathophysiological mechanism of post-infectious FD is now postulated in a subset of patients.[12] More than half of the panelist doctors excluded patients with reflux symptoms when they diagnosed FD in clinical practice, although some overlap has been reported.[13] In contrast, the majority of doctors usually included patients suggesting IBS with lower GI symptoms in FD because there is actually a significant overlap between the two disorders.[14]

Psychological tests for FD or IBS patients were not common (about 26–29%), and many doctors judged the patient's psychological condition through a routine interview. GI motility or visceral hypersensitivity was also not routinely examined even in the institutes of panelist doctors, although the importance of these examinations is recognized in consideration of the pathophysiologic mechanism.[15,16]

For medical treatment of FD, anti-secretory drugs such as H2RA and PPI were widely used by panelist doctors. PPI were prescribed to about 90% of FD patients. A meta-analysis demonstrated that PPI were more effective than a placebo for management of patients with ulcer-like and reflux-like FD,[17] and a recent study has been shown that the prevalence of pathologic acid reflux is approximately 50% in FD with epigastric burning.[18] However, the use of PPI may be limited, because there was a report that lansoprazole treatment was not superior to a placebo for management of FD in Chinese patients.[19] Prokinetics may be effective for symptom relief in some FD patients. A meta-analysis indicates that prokinetic agents were significantly more effective than a placebo in the treatment of FD, and it was also suggested that mosapride citrate may be more effective than cisapride for the treatment of FD.[20] It was also shown that mosapride citrate and famotidine (H2RA) had beneficial effects regardless of FD subtype, age and gender.[21] The effectiveness of PPI therapy and that of prokinetic therapy by mosapride citrate for FD were also reported to be not different, and cannot be predicted by Rome III subgroups.[22] In this study, all of the panelist doctors were using some prokinetics including mosapride citrate, especially for PDS subgroups, in accordance with those reported observations.

For medical treatment of IBS, about 62% of the panelist doctors usually give prescriptions to patients with IBS at the first visit. Although treatment options for IBS-C, IBS-D, and abdominal pain appear to be different,[23] polycarbophil calcium, trimebutine maleate and probiotics were commonly used, especially in Japan. Ramosetoron hydrochloride is a serotonin H3 receptor antagonist that was developed in Japan and is currently used widely for IBS-D treatment, especially in Japan.[24] As complementary alterative therapies, Chinese herbal therapy is very popular in Japan, Rikkunshito for FD and Daikenchuto for IBS, mainly because standardized forms of herbal medicine with regards to quality and quantities of ingredients are commercially available in Japan.[25]

Although results of some studies have suggested the effectiveness of anxiolytic or anti-depressive agents for treatment of FD and IBS,[26,27] these results are not sufficient to prove their benefit.[28] A recent meta-analysis has shown that the relative risk (RR) of IBS symptoms persisting with anti-depressants versus placebo was 0.66, with similar treatment effects for both tricyclic antidepressants and SSRI.[29] Although this survey showed that treatment of FGIDs with psychological drugs appears to be very popular, high-quality, larger studies in the A-P region are needed to determine whether anxiolytic or antidepressant drugs can relieve FD or IBS symptoms. On the other hand, psychotherapy was not so popular among physicians, mainly due to the difficulty in performing psychotherapy in outpatient clinics. However, since psychotherapy can sometimes be effective for chronic patients who do not respond to medical treatments,[30,31] it should be further considered in refractory cases.

From our survey, we were able to obtain valuable information about current clinical practice for diagnosis and management of FD and IBS in the A-P region. The data will be useful for the establishment of consensus guidelines for these disorders.

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Interferon-α increases monocyte migration via platelet–monocyte interaction in murine intestinal microvessels

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Summary

The aim of this study was to investigate the effect of interferon (IFN)-α on recruitment of platelets and monocytes within the murine small intestinal venular endothelium. Monocytes were isolated from bone marrow of C57B6 mice. Platelets were collected from murine blood. Rolling and adhesion to submucosal microvessels in the small intestine were examined under an intravital fluorescence microscope after injection of fluorescein-labelled monocytes or platelets. In some mice, IFN-α (5×10^5U/kg) was administered intraperitoneally. After treatment with an antibody against P-selectin, changes in monocyte and platelet migration were also investigated. Changes in monocyte migration under the condition of thrombocytopenia were also investigated. Platelets and monocytes interacted with murine intestinal microvessels, although only few platelets and monocytes showed migration behaviour. Intraperitoneal injection of IFN-α enhanced the migration of both platelets and monocytes in the intestinal microvessels. Pretreatment with anti-P-selectin attenuated the increase in migration of platelets and monocytes induced by administration of IFN-α. Thrombocytopenia decreased the rolling ratio of monocytes, suggesting that the effect of IFN-α on migration was P-selectin-dependent, derived from both the endothelium of microvessels and platelets. The results of this study suggest that IFN-α acts as a potent proinflammatory agent via its stimulatory effect on the endothelium–platelet–monocyte interaction in intestinal microvessels by a P-selectin-dependent mechanism.

Keywords: ileum, inflammation, interferon, mice, monocytes, platelets

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Introduction

Interferon (IFN)-α has several biological actions, including activation of macrophages, decrease in division of tumour cells and anti-viral activity. IFN-α is used for treatment of diseases with diverse pathogeneses and manifestations, including chronic viral hepatitis B or C, chronic myelogenous leukaemia, hairy cell leukaemia, Kaposi's sarcoma, laryngeal and genital papillomas and various angiogenic diseases [1]. IFN-α is a multi-functional immunomodulatory cytokine and its mechanism is thought to be mediated by regulating and down-modulating T helper type 2 (Th2) cytokines [2,3]. IFN-α has been shown to be effective for treatment of ulcerative colitis (UC) [4,5]. However, some prospective studies have shown that IFN-α was not effective for treatment of inflammatory bowel disease (IBD), and induction and exacerbation of UC or Crohn's disease (CD) by IFN-α during treatment of chronic hepatitis C have also been reported. Thus, the immunological effect of IFN-α on IBD is still a controversial issue. Various side effects and toxicities of IFN-α have been observed in all clinical studies to date. Retinal haemorrhage [6] and myocardial infarction [7,8] have been reported as adverse effects. Guyer *et al.* reported cases of retinal ischaemic retinopathy, characterized by cotton-wool spot formation, capillary non-perfusion, arteriolar occlusion and haemorrhage, associated with the use of IFN-α. In addition, we have reported previously that IFN-α increased leucocyte–endothelial interaction in the mesenteric microcirculation of rats *in vivo*, suggesting that IFN-α has a proinflammatory effect by increasing leucocyte recruitment. We have reported previously *in vivo* behaviour of monocyte migration in the murine intestinal mucosa [9], and that blockade of monocyte migration to the intestine ameliorated inflammation in

experimental chronic ileitis [10]. Recently, we have shown that platelets contribute to the inflammatory condition in which monocytes are involved via platelet–monocyte interaction in lipopolysaccharide (LPS)-induced acute ileitis [11]. In addition, we demonstrated that control of platelet recruitment ameliorates chronic murine ileitis by decreasing monocyte migration [12]. Because thrombocytopenia is usually seen in patients treated with IFN, we hypothesized that IFN enhances platelet–endothelial interaction, evoking a proinflammatory effect of monocytes by increasing monocyte recruitment to the intestinal mucosa. The objective of this study was to assess the influence of IFN-α on microcirculation in the small intestine, focusing on platelet and monocyte interactions with the venular endothelium.

Materials and methods

Animals

Male C57B6 mice, 8–10 weeks old (Clea Japan, Tokyo Japan), were maintained on standard laboratory chow (SLC, Tokyo, Japan) and in specific pathogen-free conditions. The care and use of laboratory animals were in accordance with the guidelines of the animal facility in National Defense Medical College (NDMC). This study protocol was approved by Animal Ethical Committee of NDMC (no. 08103).

Isolation of monocytes and plateles and labelling with carboxyfluorescein diacetate succinimidyl ester (CFSDE)

Monocytes were isolated from the bone marrow of murine thigh and labelled as described previously [11,12]. Briefly, bone marrow cells were obtained from thigh bone of C57B6 mice and monocytes were isolated by magnetic activated cell sorting (MACS; Miltenyi Biotec, Auburn, CA, USA) with beads-conjugated anti-rabbit CD11b polyclonal antibody (Miltenyi Biotec). The purity of monocytes and uniformity of the isolation procedure were compared between batches by a fluorescence-activated cell sorter (FACSCalibur; Becton-Dickinson, Mountain View, CA, USA) using rabbit anti-mouse CD14 polyclonal antibody (Santa Cruz Biotec, Santa Cruz, CA, USA) and confirmed that approximately 94% of $CD11b^+$ cells from each batch expressed CD14. Platelets were isolated from blood of donor mice, as described previously (H26, H27 [13,14]). Blood from the mice was collected from the heart and platelets were isolated by centrifugation at 600 *g* with 0·1 ml acid citrate dextrose buffer. The expression of P-selectin on platelets was compared between batches by FACS using rat anti-mouse P-selectin (RB40.34; BD PharMingen, San Diego, CA, USA) and confirmed that expression of P-selectin did not differ between batches. CFDSE (Molecular Probes, Eugene, OR, USA) was dissolved in dimethylsulphoxide at 15·6 mM, divided into small aliquots (each 300 μl), and stored in a cuvette sealed with argon gas at −20°C until experimental use. Monocytes (approximately 2×10^7) in 1·5 ml of phosphate-buffered saline (PBS) were incubated with CFDSE solution for 10 min at 4°C and washed with PBS. Platelets (approximately 1×10^8) were incubated with CFDSE solution for 10 min at 4°C and washed with PBS.

Animal preparation for intravital observation

For migration studies, mice were anaesthetized with 50 mg/kg pentobarbital sodium, and the abdomen of each mouse was opened with a midline incision. An ileal segment 1–3 cm in length was selected for observation. The intestine was kept warm and moist by continuous superfusion with PBS warmed to 37°C. PBS was injected into the selected segment using a 30-gauge needle. The behaviour of monocytes and platelets in submucosal venules was observed from the serosal side using an intravital microscope. The behaviour of CFDSE-labelled monocytes and platelets was visualized on a monitor through a silicon-intensified target image tube (SIT) system, using a previously described method, and recorded on a digital hard disk recorder [4]. Microcirculation was observed by fluorescence microscope (BX51WI; Olympus, Tokyo, Japan) equipped with a contrast-enhancing unit (C-2400-08; Hamamatsu Photonics, Shizuoka, Japan) and ×10 ultraviolet-fluorite objective lens (Uplan Fl; Olympus, Tokyo, Japan).

Administration of IFN-α and monoclonal antibodies

IFN-α (5×10^5 U/Kg) was administered intraperitoneally 2 h before CFDSE-labelled monocytes or platelets were injected via a cervical vein. In some experiments, 2 mg/kg of rat anti-mouse P-selectin (RB40.34; BD PharMingen, San Diego, CA, USA) was administered via a cervical vein 5 min prior to CFDSE-labelled monocyte administration [9–11,15]. To induce thrombocytopenia, mice were injected intraperitoneally with GPIbα (CD42b) monoclonal antibody (EMFRET Analytics, Würzburg, Germany) with 80 μg in 200 μl PBS 24 h before the initiation of the experiment [11]. This treatment induced severe thrombocytopenia.

Analysis of monocyte and platelet dynamics

The number of adherent monocytes was counted off-line by digital video disk images. Monocytes adhering to vascular walls with or without occasional movements were defined as rolling monocytes. In these rolling monocytes, those which were adhering to vascular walls without movement and remaining stationary for a period of more than 30 s were defined as adherent monocytes. We counted the numbers of total influx, rolling monocytes and adherent monocytes using a ×10 objective lens for 30 min after the injection. The rolling rate was defined as the ratio of rolling monocytes divided by total monocytes and the adhering number was

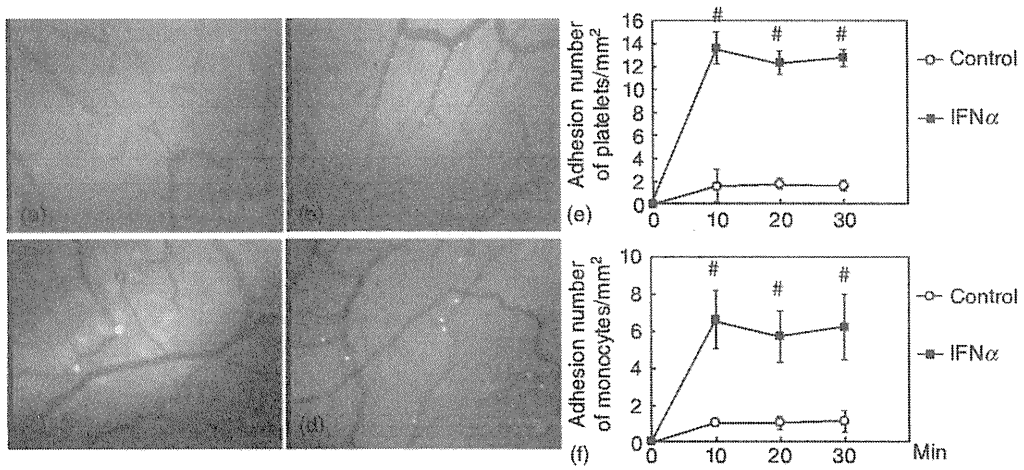

Fig. 1. Effect of interferon (IFN)-α on migration of platelets and monocytes. Representative photos of *in vivo* observation of platelets migration in intestinal microvessels (a,b). In control mice, a small number of labelled platelets adhered to submucosal venules (a). In IFN-α-treated mice, the number of adherent platelets increased significantly (b). Representative photos of *in vivo* observation of monocyte migration in intestinal microvessels (c,d). In control mice, a small number of labelled monocytes adhered to submucosal venules (c). In IFN-α-treated mice, the number of adherent monocytes increased significantly (d). Time–course of platelets adhesion in the small intestine submucosal venules. IFN-α increased platelets adhesion significantly (e). Time–course of monocytes adhesion in the small intestine submucosal venules. IFN-α increased monocytes adhesion significantly (f). ○: Control; ■: IFN-α treated group; #$P < 0{\cdot}05$ *versus* control.

defined as count number of adherent monocytes in 1 mm^2. Those platelets adhering to vascular walls without movement and remaining stationary for a period of more than 30 s were defined as adherent platelets. We counted the number of adherent platelets in a 1 mm^2 field. In another set of experiments platelets were labelled with CFDSE and monocytes were labelled with rhodamine G. These platelets and monocytes were infused together into recipient mice and the interaction between platelets and monocytes was observed simultaneously by 3CCD camera (VB-7010; Keyence, Osaka, Japan), ×10 ultraviolet-fluorite objective lens (Uplan Fl; Olympus, Tokyo, Japan) and fluorescence mirror unit (UIS2; Olympus, Tokyo, Japan).

Statistics

All results are expressed as means ± standard errors (s.e.) of four or five mice. For comparison of rolling and adhesion, the mean values were evaluated statistically by a non-parametric Mann–Whitney *U*-test. Statistical significance was defined as $P < 0{\cdot}05$.

Results

Interaction of platelets and monocytes with intestinal microvessels

Figure 1a shows *in vivo* images of fluorescence-labelled platelets in intestinal submucosal microvessels in the control condition obtained using an intravital fluorescein microscope. In the control uninflamed intestine, only a few platelets showed the characteristic rolling behaviour on the surface of microvessels.

Figure 1b shows *in vivo* images of platelets in IFN-α-treated intestinal submucosal microvessels. The number of rolling platelets increased significantly in IFN-α treated mice. The microvessel diameters were not changed by IFN-α treatment. Figure 1e shows the platelet adhesion time–course to the small intestine microvessels. IFN-α treatment increased the number of adherent platelets significantly (approximately 12–14/mm^2) compared with the control group.

Figure 1c shows *in vivo* images of fluorescence-labelled monocytes in intestinal submucosal microvessels in the control condition obtained by using an intravital fluorescein microscope. In the control uninflamed intestine, only a few monocytes showed characteristic rolling behaviour on the microvessel surface and the monocyte rolling ratio was approximately 21% (Table 1). Figure 1f shows the time–

Table 1. The rolling ratio of fluorescence-labelled monocytes in submucosal venules of small intestine.

	Rolling ratio (%)
Control	21 ± 2
IFN-α	42 ± 2#
IFN-α + anti-P-selectin	21 ± 3*
IFN-α + anti-platelet	30 ± 2*

Effect of interferon (IFN)-α and monoclonal antibodies. Rolling monocytes were counted for 30 min after infusion of 5,6-carboxy-succinimidyl fluorescein-ester (CFSE)-labelled monocytes. Rolling ratio was expressed as percentage of total number of monocytes appearing. #$P < 0{\cdot}05$ *versus* control; *$P < 0{\cdot}05$ *versus* IFN-α-treated group.

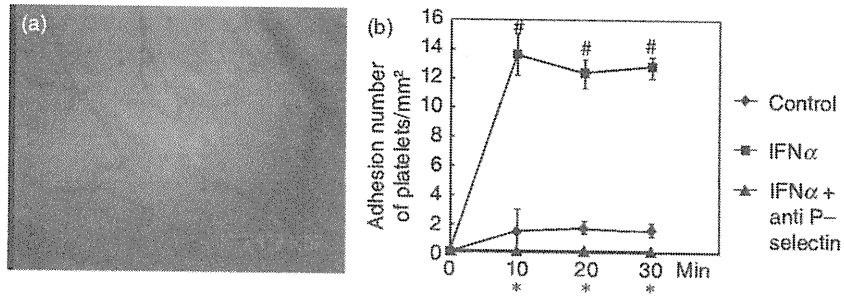

Fig. 2. Effect of the anti-P-selectin antibody treatment on enhanced platelet migration which was induced by interferon (IFN)-α treatment. Representative photographs of *in vivo* observation of platelet migration in intestinal microvessels. The anti-P-selectin antibody treatment to IFN-α-treated animals blocked migration behaviour of platelets (a). Time–course of platelets adhesion in the small intestine submucosal venules. IFN-α increased platelets adhesion significantly. Anti P-selectin antibody attenuated the increase of migration of platelets induced by IFN-α. (b). ○: Control; ■: IFN-α-treated group; ▲: IFN-α + anti-P-selectin antibody-treated group. #$P < 0{\cdot}05$ *versus* control; *$P < 0{\cdot}05$ *versus* IFN-α-treated group.

course of monocyte adhesion to the small intestine microvessels. Under the IFN-α treatment condition, the number of adherent monocytes also increased significantly (Fig. 1d,f). The monocyte rolling ratio also increased significantly to approximately 42% (Table 1). The increase in rolling fluorescein-labelled monocytes was observed 10 min after infusion and continued during the observation period.

Effect of pretreatment with anti-adhesion molecules on IFN-α-induced platelet adhesion

To investigate the mechanism by which platelet adhesion to intestinal microvessels is enhanced by IFN-α, the effect of an anti-P-selectin antibody was investigated. Fig. 2a shows the effects of pretreatment with anti-P-selectin monoclonal antibody (mAb) on enhanced platelet migration. As shown in this image, the number of adherent platelets was decreased significantly by pretreatment with anti-P-selectin mAb. The time–course of the effect by anti-P-selectin on platelet adhesion is shown in Fig. 2b. Enhanced platelet adhesion by IFN-α was decreased significantly by pretreatment with anti-P-selectin mAb and reached basal levels, showing that IFN-α-induced platelet adhesion to intestinal microvessels was dependent on P-selectin.

Effect of pretreatment with anti-adhesion molecules on IFN-α-induced monocyte migration

We have reported previously that platelets induced monocyte recruitment to the vascular endothelium by interaction between P-selectin on platelets and PSGl-1 on monocytes [11]. To clarify the platelet involvement in monocyte migration to intestinal microvessels, we examined further whether controlling platelet adhesion could attenuate monocyte migration to intestinal microvessels. For this purpose, the interaction of monocytes with microvessels was observed after treatment with anti-P-selectin mAb, which successfully attenuated IFN-α-induced platelet adhesion to the venular endothelium. As shown in Fig. 3a, IFN-α-induced enhancement of monocyte migration was attenuated by anti-P-selectin mAb. As shown in Fig. 3b, pretreatment with anti-P-selectin antibody attenuated significantly IFN-α-induced monocyte adhesion to intestinal microvessels. Table 1 shows the effects of pretreatment with these mAbs on the monocyte

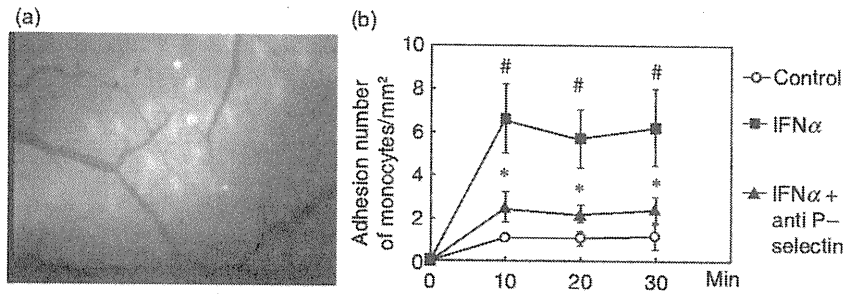

Fig. 3. Effect of the anti-P-selectin antibody treatment on enhanced monocyte migration which was induced by interferon (IFN)-α treatment. Representative photographs of *in vivo* observation of monocyte migration in intestinal microvessels. The anti-P-selectin antibody treatment to IFN-α-treated animals blocked monocyte migration behaviour (a). Time–course of monocyte adhesion in the submucosal venules of small intestine. IFN-α increased monocyte adhesion significantly. Anti P-selectin antibody attenuated the increase of migration of monocytes induced by IFN-α. (b). ○: Control; ■: IFN-α treated group; ▲: IFN-α + anti P-selectin antibody-treated group; #$P < 0{\cdot}05$ *versus* control; *$P < 0{\cdot}05$ *versus* IFN-α-treated group.

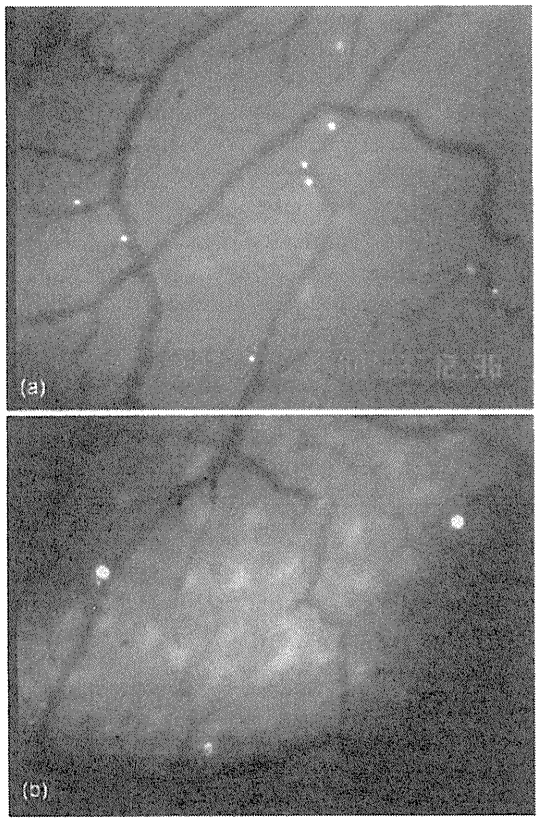

Fig. 4. Effect of thrombocytepenia by anti-platelet antibody treatment on enhanced monocyte migration which was induced by interferon (IFN)-α treatment. Representative photographs of *in vivo* observation of monocyte migration in intestinal microvessels. In IFN-α-treated mice, the number of adherent monocytes increased significantly (a). The anti-platelet antibody treatment to IFN-α-treated animals attenuated the number of monocyte migration (b).

rolling ratio. Anti-P-selectin attenuated significantly the monocyte rolling ratio induced by IFN-α from approximately 42% to 21%. These findings suggest that platelet-derived P-selectin and its ligand on monocytes are also involved in this mechanism.

Effect of thrombocytopenia on IFN-α-induced monocyte migration

To clarify further the involvement of platelets in monocyte migration to intestinal microvessels, we examined whether a decrease in the number of platelets could attenuate monocyte migration to intestinal microvessels. For this purpose, the interaction of monocytes with microvessels was observed after treatment with anti-platelet mAb (GPIbα antibody). Treatment with anti-platelet mAb (GPIbα antibody) dramatically decreased the number of platelets from $105 \pm 10 \times 10^4$ to $3{\cdot}8 \pm 1{\cdot}2 \times 10^4$. As shown in Fig. 4, IFN-α-induced enhancement of monocyte migration was attenuated by anti-platelet mAb. Table 1 shows the effects of pretreatment with these mAbs on the monocyte rolling ratio. Induction of thrombocytopenia by anti-platelet mAb administration decreased the monocyte rolling ratio significantly from approximately 42% to approximately 30%. These findings suggest that platelets are involved in the mechanism of IFN-α-induced monocyte adhesion.

Discussion

This study provided *in vivo* evidence of the induction of interaction between monocytes, platelets and endothelium on microvessels of the murine small intestine by administration of IFN-α.

Accumulating evidence indicates recruitment of monocytes on the vessel wall either through a direct interaction with endothelial cells in the absence of platelets (monocytes–endothelium interaction) or indirectly by binding with platelets through PSGl-1–P-selectin interaction (monocytes–platelets interaction [16]. We have reported previously that inhibition of platelet migration attenuated monocyte migration induced by LPS in intestinal microvessels [11], suggesting that platelets on the inflamed endothelium of microvessels play an important role in monocyte migration. In addition, we reported that inhibition of platelet migration ameliorated murine ileitis by blocking the recruitment of monocytes to the inflamed intestinal mucosa.

In this study, we found that the rolling and adhesion of platelets and monocytes were increased significantly after IFN-α intraperitoneal administration, and thrombocytopenia induced by anti-platelet antibody (GPIbα antibody) decreased the monocyte rolling ratio. Collectively, these findings suggest that interaction between platelets and monocytes are involved in enhanced monocyte recruitment by treatment of IFN-α. In this study, anti-platelet mAb induced an approximately 96% decrease in the number of platelets. However, the increased rolling by IFN-α treatment was attenuated partially by this treatment. This suggests that monocytes also recruit on the vessel wall through a direct interaction with endothelial cells in the absence of platelets (monocytes–endothelium interaction). Consistent with this, anti-P selectin antibody treatment, which blocks both endothelium-derived P-selectin and platelet-derived P-selectin, completely blocked monocyte recruitment. Collectively, these findings suggest that the effect of IFN-α on monocyte migration was P-selectin-dependent, derived not only from platelets but also from the endothelium of microvessels. In our preliminary study, we investigated the surface expression of P-selectin on platelets and that of PSGl-1 on monocytes after incubation with IFN-α *in vitro* by flow cytometry. Expression of both surface adhesion molecules remained unchanged after IFN-α treatment, consistent with results of a previous study [17]. It was also shown in that study [17] that IFN-α activates the vascular endothelium, as assessed by von Willebrand factor antigen levels [17]. Taken together, the results suggest that IFN-α initially induces an increase in the expression of

P-selectin on the vascular endothelium, resulting in the enhancement of rolling platelets and monocytes. Some monocytes interacted with platelets and they displayed rolling behaviour efficiently.

IFNs are used to treat a variety of diseases, including renal cell carcinoma, viral hepatitis and other malignant diseases. The doses of IFN-α used for treatment of viral hepatitis and renal cell carcinoma are $3–6 \times 10^6$ IU/m^2 [18] and $6–24 \times 10^6$ IU/m^2 [19], respectively. Previous clinical studies have revealed that IFN-α at a dose of more than 36×10^6 IU induces severe toxicity and significantly alters the patient performance [20]. It is noteworthy that the IFN-α dosage used in the present study was equivalent to that used in humans. In our preliminary experiment, we investigated whether increased monocyte migration induced by IFN-α treatment injured the vascular endothelium. Albumin leakage from venules to the extravascular area, however, was not increased significantly by IFN-α treatment (data not shown). Thus, in the healthy endothelium, some protective mechanisms of the host might overcome the effect of aberrant monocyte migration induced by IFN-α. However, it is generally accepted that damaged vascular endothelium under inflamed conditions increases surface expression of adhesion molecules. Thus, it is possible that IFN-α has a proinflammatory effect under pathlogical conditions through increasing monocyte migration.

Cytokines play a key role to determine differentiation of T cells into Th1, Th2, T regulatory and Th17 cells. It is now generally accepted that the two forms of IBD are associated with distinct immune profiles, which are classified as a fairly typical Th1 response in CD and an atypical Th2 response in UC [21]. Based on these findings, in some studies IFN-α has been used for treatment of UC [4,5,22]. IFNs are cytokines possessing immunoregulatory properties and have been used to treat a number of chronic inflammatory disorders successfully, including chronic viral hepatitis B or C, chronic myelogenous leukaemia, hairy cell leukaemia, Kaposi's sarcoma, laryngeal and genital papillomas and various angiogenic diseases [1]. However, IFN-α is known to worsen several diseases linked to Th1-mediated pathophysiology such as psoriasis and arthritis [1]. Two studies showed that IFN-α was effective for treatment of UC, while another study showed that it was not effective. In addition, induction or exacerbation of UC during treatment of chronic hepatitis C by IFN-α has been reported [23–26]. Based on these reports, the effectiveness of IFN-α for treatment of UC as a Th2-linked disease remains unclear. Furthermore, for CD, only one clinical trial using IFN-α has been performed and the results showed no therapeutic effectiveness [27]. Although the mechanism of action of IFN-α remains to be elucidated, it is difficult to accept IFN-α as a drug for treatment of IBD [28].

In this study, adhesion of platelets increased significantly after administration of IFN-α, suggesting the promotion of P-selectin expression on the microvessel endothelium in the small intestine in a few hours. Although the mechanism of the increase in P-selectin expression remains unclear, IFN-α seems to have a proinflammatory effect in a normal immunological state in the small intestine. Although, as mentioned above, some clinical trials did not demonstrate the effectiveness of IFN-α, the present study suggests that IFN-α acts as a potent proinflammatory agent via its stimulatory effect on endothelium–platelet–monocyte interaction in intestinal microvessels, and the results of our study may provide basic information to explain why IFN-α has a proinflammatory role in the intestinal mucosa.

Disclosure

There is no personal or financial conflict of interest to disclose for any of the authors listed.

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Chronic nonspecific multiple ulcer of the small intestine segregates in offspring from consanguinity

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Abstract

Background and aims: Chronic nonspecific multiple ulcer of the small intestine is a recently proposed enteropathy characterized by persistent blood and protein loss from the small-bowel. We examined possible segregation of the disease in family pedigrees.
Methods: All cases of the disease diagnosed at our institution were reviewed with respect to particular focuses on the presence of close consanguinity in the families, the enteroscopic findings and the long-term clinical course. The diagnosis was based on persistent occult gastrointestinal bleeding and hypoproteinemia for more than 5 years, and irregularly shaped shallow ulcers in the ileum.
Results: During a 45-year-period, 13 patients were diagnosed as having the disease. There were 11 females and 2 males, with ages ranging from 8 to 37 years at the time of the initial presentation and with those from 13 to 38 years at the diagnosis. Enteroscopy performed in 11 patients with a time duration ranging from 0.5 to 44 years after the diagnosis revealed active ileal ulcers in 10 patients. Parents' consanguineous marriage was verified in 6 patients, two of whom also had siblings with the enteropathy. Another patient without consanguinity had a sibling with protein-losing enteropathy.
Conclusion: Chronic nonspecific multiple ulcer of the small intestine seems to segregate in offspring from consanguineous marriage.

1. Introduction

The use of capsule endoscopy and balloon endoscopy has led to an increase in the chance of encountering small-bowel ulcers, especially in patients with obscure gastrointestinal bleeding.[1,2] While Crohn's disease, intestinal tuberculosis,

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radiation enteropathy, and nonsteroidal anti-inflammatory drug (NSAID) enteropathy are entities predisposing to chronic or recurrent small-bowel ulcers, there are cases of ulcers with obscure origin.

We recently reported on a peculiar form of enteropathy characterized by chronic blood and protein loss through persistent small-bowel ulcers.[3] Because the ulcers of the disease had nonspecific histology, we referred to the condition as "chronic nonspecific multiple ulcer of the small intestine (CNSU)".[3,4] CNSU does not seem to be a rare entity, because cases of exactly the same clinicopathologic features have subsequently been reported in the literature.[5-7] Furthermore, a similar enteropathy with different nomenclatures has been described in Caucasians and referred to as "diaphragm disease of the small bowel without apparent NSAID use"[8] or as "cryptogenic multifocal ulcerous stenosing enteritis".[9] More recently, Adler et al.[10] reported a novel enteropathy in a middle aged American male characterized by blood loss from recurrent small-bowel ulcers. Surprisingly, Adler's case had compound heterozygous mutations in the encoding regions of *cytosolic phospholipase A2α (cPLA2α)* gene. Based on the description, we hypothesized CNSU to be a hereditary condition with genetic alterations. We thus retrospectively investigated family histories of CNSU in patients with the disease identified at our institution.

2. Patients and methods

2.1. Survey for CNSU

We reviewed the diagnosis, the prevalence, and the management of inflammatory bowel diseases diagnosed during a period 1964–2009 at Kyushu University Hospital, Fukuoka University Chikushi Hospital, and their satellite hospitals, and collected data for clinicopathologic features of patients with CNSU. The two referral centers have been treating approximately 600 patients with Crohn's disease and 800 patients with ulcerative colitis.

2.2. Diagnosis of CNSU

The diagnosis of CNSU was made on the basis of clinical manifestations and small-bowel lesions.[4] As for clinical manifestations, patients with CNSU should have iron deficiency anemia and hypoproteinemia in their adolescence.[4] Small-bowel lesions should be multiple shallow ulcers in the ileum, with sharply demarcated margin and linear or oblique configuration (Fig. 1).[11] Furthermore, the repeated ascertainment of those clinical manifestations with time intervals for more than 5 years was inevitable for the diagnosis of CNSU.

2.3. Data collection

We focused on the demographic data regarding the initial clinical manifestation, which led to the identification of small-bowel ulcers, the age at the onset, and the laboratory values of serum protein, serum albumin, C-reactive protein (CRP), hemoglobin, and white blood cell count at the time of the initial diagnosis. We also reviewed histories and laboratory data presumably associated with other enteropathy. They included history of NSAID use, purified protein derivative (PPD) skin test, interferon-γ assays (IGRA) for *Mycobacterium* infection, anti-tissue transglutaminase (tTGA) antibodies, findings obtained by esophagogastroduodenoscopy with forceps biopsy, and histologic findings of the resected small bowel. In addition, medical and surgical treatments, response to the medication as determined by changes in serum protein value, and prognosis were retrospectively investigated. We also collected data of the final enteroscopic findings. The procedures for enteroscopy included retrograde ileoscopy (RI), double balloon endoscopy (DBE) and intraoperative endoscopy (IOE). The enteroscopic findings were evaluated with regard to the stage (open or scarred), the depth (deep or shallow), and the configuration (circular, linear, or their combination) of the representative lesion.[11]

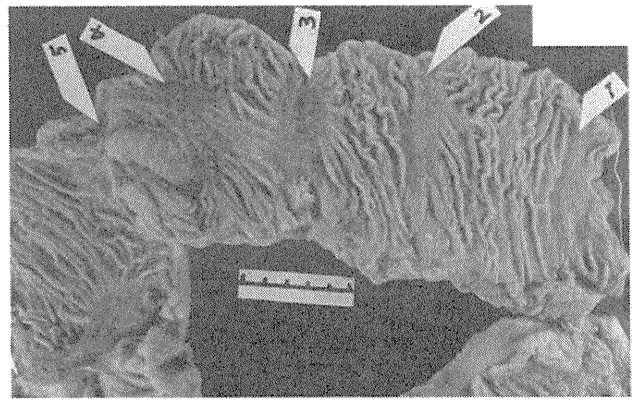

Figure 1 Typical macroscopic findings of the resected ileum in a case of CNSU (Case 9). There are shallow and clear ulcers in circular or linear configuration in the ileum. The intervening mucosa is not affected.

We directly contacted the patients and/or their relatives to obtain family histories. The items of special interest were consanguinity, anemia, malnutrition, abdominal surgery, and clinical diagnosis of enteropathy, if any, in the family pedigrees. Family history of enteropathy was regarded as positive in the case of surgical interventions for the small bowel, the established diagnosis of small-bowel ulcers or both. We examined the medical records of the relatives with enteropathy in the case that the records were available.

This retrospective study was approved by the ethical committee at Kyushu University Hospital, and it was undertaken in accordance with Helsinki Declaration.

3. Results

3.1. Clinical features and laboratory data

During a period from 1964 to 2009, 13 patients were diagnosed with CNSU. Table 1 summarizes the clinical features of the patients. There were 11 females and two males. All patients had anemia of obscure origin as the presenting symptom. In addition, three patients had edema and other two patients complained of abdominal pain. The age at the time of the onset ranged from 8 to 37 years. Eleven patients complained of the symptoms at the age of less than 20 years. The time interval

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Table 1 Cases of CNSU diagnosed at our institution during 1964–2009.

Case no.	Age (yrs)/gender		Presenting symptoms	Laboratory data		
	Onset	Diagnosis of CNSU		Hemoglobin (g/dl)	Serum protein (g/dl)	CRP (mg/dl)
1.	20/F	27	Anemia, edema	8.2	4.9	–*
2.	15/F	24	Anemia, edema	3.5	5.0	–*
3.	10/M	26	Anemia, growth retardation	4.4	4.5	–*
4.	15/F	28	Anemia, edema	4.7	5.3	–*
5.	12/F	27	Anemia, abdominal pain	9.7	5.8	0.3
6.	17/F	34	Anemia	9.6	4.6	0.5
7.	10/F	13	Anemia, abdominal pain	7.4	5.4	0.1
8.	37/F	38	Anemia	9.5	6.7	0.5
9.	15/M	30	Anemia, edema	7.4	8.2	0.1
10.	13/F	29	Anemia	5.9	4.6	0.2
11.	16/F	52	Anemia	5.3	6.3	0.1
12.	13/F	40	Anemia	9.4	4.1	1.1
13.	8/F	33	Anemia, edema	8.6	4.5	0.6

* CRP was determined to be negative under semi-quantitative measurement.

from the onset until diagnosis of CNSU ranged from 1 to 27 years (median; 15 years). NSAID use was not verified in any patient at the time of the initial diagnosis. We further confirmed possible use of NSAID in seven patients who had been under observation. Those patients again clearly denied any continuous use of NSAID or other medications at the time of their first diagnosis of CNSU.

Laboratory data at the initial diagnosis showed hypochromic anemia and hypoproteinemia. The hemoglobin value ranged from 3.5 to 9.7 g/dl and serum protein value from 4.1 to 8.2 g/dl. In four patients (Cases 1–4) with the diagnosis of CNSU in 1970s, CRP value was not quantified. In the remaining nine patients, there were slight increases in CRP with values from 0.1 to 1.1 mg/dl.

Eleven patients were treated by surgery. The remaining two patients (Cases 11 and 13) were diagnosed with CNSU on the basis of the clinical and enteroscopic findings. Results of the diagnostic work-up are summarized in Table 2. PPD skin test and IGRA showed none of the patients to be positive for *Mycobacterium* infection. Anti-tTGA antibodies were measured

Table 2 Results of diagnostic work up for patients with CNSU.

Case no.	PPD skin test	IGRA test	Anti-tTGA antobody	Gastroduodenal lesions			Surgically removed ileal lesions			Final enteroscopic findings			
										Concentric stenosis		Non-stricturing ulcers	
				Endoscopy	Granuloma	Villous atrophy	Maximal depth of ulcer	Granuloma	Villous atrophy	Number	Open ulcer at stenosis	Circular	Linear
1.	–	NE	NE	NS	–	–	Submucosa	–	–	NE	NE	NE	NE
2.	–	NE	NE	Gastric ulcer	–	–	Submucosa	–	–	Multiple	+	+	+
3.	–	NE	NE	NS	–	–	Submucosa	–	–	NE	NE	NE	NE
4.	–	NE	NE	NS	–	–	Submucosa	–	–	Single	+	–	–
5.	+	–	NE	Duodenal ulcer	–	–	Submucosa	–	–	NE	NE	NE	NE
6.	+	–	NE	NS	–	–	Submucosa	–	–	Multiple	+	+	+
7.	–	–	NE	NS	–	–	Submucosa	–	–	Single	+	+	–
8.	±	–	NE	Stomal ulcer	–	–	Submucosa	–	–	-		+	+
9.	–	NE	NE	Stomal ulcer	–	–	Submucosa	–	–	Single	+	+	–
10.	–	NE	NE	NS	–	–	Submucosa	–	–	Multiple	+	+	+
11.	–	NE	NE	NS	–	–	NE	NE	NE	Single	+	–	–
12.	–	NE	NE	NS	–	–	Submucosa	–	–	Multiple	+	–	+
13.	–	–	–	NS	–	–	NE	NE	NE	Single	+	–	+

PPD; purified protein derivative. IGRA; interferon-γ release assays for tuberculosis. tTGA; tissue transglutaminase.
NS; no significant finding. NE; not examined.

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in only one patient, who showed a negative result. Two patients had a prior history of gastrectomy for gastroduodenal ulcers. Both patients had stomal ulcers. Two patients had gastric or duodenal ulcer. However, duodenal biopsies performed in all the patients were negative for villous atrophy. Also, villous atrophy of the ileum was not evident in any patient treated by ileal resection. The depth of the ileal ulcer was restricted to the submucosa in those patients. There was not any patient who had caseating or non-caseating granuloma in the biopsy or surgical specimens.

Table 3 summarizes the treatments applied for the patients. During the follow-up periods, prednisolone, aminosalicylates, combined anti-*Mycobacterium* agents, azathioprine and infliximab were used for nine patients, seven patients, six patients, two patients and a patient, respectively. The serum protein did not respond to any of those medications. In nine patients, the malnutrition transiently improved after total parenteral nutrition. Eleven patients were treated by ileal resection because of small-bowel stricture. Ten of those 11 patients, however, required repeated surgery after the recurrence of strictures. As indicated in Table 3, two patients were lost to follow up, while other four patients died. The remaining seven patients have been under observation. They still have hypoproteinemia and anemia, which require iron supplementation and total parenteral or enteral nutrition.

3.2. Final enteroscopic findings

We attempted enteroscopy in 11 patients during the clinical course. The time interval from the initial diagnosis until the final enteroscopy ranged from 0.5 to 44 years. In a patient (Case 5), however, enteroscopy was unavailable because of a duodenal stenosis.

The enteroscopic findings are indicated in Table 2. Nine patients had single or multiple concentric stenoses. In those patients, shallow and clearly demarcated ulcers were seen at the most severe stenosis (Figs. 2A and 3A). In addition, shallow ulcers accompanied by faint mucous exudates were seen in eight patients (Figs. 2B and 3B). A patient had a single stenosis without any accompanying mucosal defects.

3.3. Family history

Family histories of the patients are indicated in Table 4. The interviews to the patients and their relatives revealed that four patients were offspring of consanguineous marriage of 3 degrees, which means that their parents were cousins. In addition, other two patients were those of 5 degrees, indicating that their maternal and paternal grandparents were cousins. Four patients denied any such consanguinity in their family pedigrees. In the remaining three patients, we were not able to confirm their family pedigrees.

Information with regard to family histories of enteropathy was available in 11 patients. None of the patients commented on enteropathy in their parents or in their offspring. However, three patients commented on enteropathy in their siblings. The enteropathy included small intestinal stenoses of obscure origin (an elder sister of Case 4), CNSU (a younger sister of Case 10) verified in her medical record, and protein-losing enteropathy of obscure origin (an elder sister in Case 13). Two of the three family pedigrees were siblings of consanguineous marriage, while consanguinity was not evident in the remaining pedigree.

4. Discussion

We could confirm in this report that 1) CNSU is an enteropathy characterized by persistent anemia and hypoproteinemia occurring in childhood or in adolescence, 2) patients with CNSU had life-long illness, and 3) more than half of the patients had consanguinity and/or family history of enteropathy in their siblings even though vertical heredity was not obvious. These clinical observations suggest that CNSU is possibly a chronic enteropathy, which segregates in offspring from consanguinity. Even though most autosomal recessive disorders of the human bowel occur in infancy,[12] there have been recently reported two gastrointestinal disorders with such a hereditary trait, one being adenomatous polyposis with homozygous mutations of *MUTYH*[13–15] and the other chronic colitis with homozygous mutations of *IL10R*.[16]

Table 3 Treatment and prognosis of patients with CNSU.

Case no.	Medication		Efficacy of total parenteral nutrition	Number of ileal resection	Prognosis
	Species	Efficacy			
1.	PSL, cAMA	Not effective	Effective	2	Lost to follow-up
2.	PSL, cAMA, SASP	Not effective	Effective	3	Died of liver cirrhosis at age of 49 years
3.	PSL, cAMA	Not effective	(Not performed)	6	Lost to follow-up
4.	PSL, cAMA, SASP	Not effective	Effective	3	Died of pancreas cancer at age of 73 years
5.	PSL	Not effective	Effective	2	Alive at age of 59 years
6.	PSL, 5ASA, AZA	Not effective	Effective	1	Alive at age of 58 years
7.	PSL, cAMA	Not effective	Effective	6	Died of thyroid cancer at age of 58 years
8.	PSL, 5ASA	Not effective	Effective	2	Alive at age of 75 years
9.	PSL, cAMA, SASP	Not effective	(Not performed)	2	Alive at age of 67 years
10.	5ASA	Not effective	Effective	2	Died of stroke at age of 46 years
11.	IFX	Not effective	(Not performed)	0	Alive at the age of 60 years
12.	(None)		Effective	3	Alive at the age of 50 years
13.	5ASA, AZA	Not effective	(Not performed)	0	Alive at the age of 35 years

PSL; prednisolone, cAMA; combined anti-*Mycobacterium* agents, SASP; sulfasalazine, 5ASA; 5-aminosalicylate, AZA; azathioprine, IFX; infliximab.

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