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Roth, Bodil; Ohlsson, Bodil

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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Short title: Gastrointestinal symptoms in MC

**Gastrointestinal symptoms and psychological well-being in patients with
microscopic colitis**

Bodil Roth and Bodil Ohlsson

Department of Clinical Sciences, Division of Gastroenterology, Skåne University

Hospital, Malmö, Lund University, Lund, Sweden

Corresponding author:

Bodil Ohlsson

Inga-Maria Nilsson gata 46

SUS Malmö

205 02 Malmö

Bodil.ohlsson@med.lu.se

Abstract

Objective. Microscopic colitis (MC) and irritable bowel syndrome (IBS) are both gastrointestinal disorders with female predominance that affect well-being. Autoantibodies against gonadotropin-releasing hormone (GnRH) have recently been detected in IBS patients. The purpose of this study was to compare gastrointestinal symptoms and well-being in MC female outpatients, with or without co-existing IBS-like symptoms, and to examine the prevalence of GnRH antibodies in these patients.

Material and Methods. Women with biopsy-verified MC, at any outpatient clinic of the Departments of Gastroenterology, Skåne, between 2002 and 2010 were invited to participate in the study. The questionnaires Gastrointestinal Symptom Rating Scale (GSRS), Psychological General Well-being Index (PGWB), Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS) and Rome III were answered and blood samples collected. Autoantibodies (IgG, IgA and IgM) against GnRH and GnRH-R (extracellular peptide of receptor) were determined by an ELISA assay.

Results. Altogether, 159 (66.2 %) of 240 invited patients with MC were recruited to the study. Of these, 134 (55.8 %) also accepted to provide blood samples. Patients with IBS-like symptoms (55%) experienced more symptoms and worse psychological well-being in all dimensions in GSRS and PGWB, and in all symptoms but constipation in VAS-IBS compared to patients without IBS symptoms. Only a minority of patients expressed antibodies against GnRH or GnRH-R, which did not differ between groups.

Conclusions. MC patients fulfilling criteria for IBS experience more gastrointestinal symptoms and worse psychological well-being than those who do not. Autoantibodies against GnRH or GnRH-R were not observed in MC patients.

Key words: Irritable bowle syndrome (IBS), Microscopic colitis (MC), quality of life, gastrointestinal symptoms, collagenous colitis (CC), lymphocytic colitis (LC)

Introduction

Microscopic colitis (MC) and irritable bowel syndrome (IBS) are both gastrointestinal disorders/disturbances that affect the patient's daily life and well-being (1, 2, 3). Many symptoms are similar for MC and IBS, like abdominal pain, bloating and change in bowel habits, but MC is characterized by watery diarrhoea, and IBS is more often associated with abdominal pain, bloating and alternating diarrhoea or constipation (4, 5). Predominance for women is true in both cases, but more pronounced in IBS. The onset of IBS occurs in younger age, e.g. 30-40 years, while in the middle age or elderly in terms of MC (6, 7).

MC encompasses two subtypes, collagenous – and lymphocytic colitis (CC and LC, respectively), and are diagnosed using histological criteria. Both subtypes present themselves with intraepithelial lymphocytosis, but CC is also accompanied by a subepithelial, thickened collagenous layer (8). There is no specific clinical test for IBS, diagnosis are made solely on symptoms according to Rome III criteria (6). A biopsy can thus differentiate MC from IBS, but as many as 56 % of the patients with MC also fulfilled the Rome II criteria for IBS (9, 10).

Aetiology and pathogenesis are not fully understood for any of the conditions, but evidence for psychologic factors, inflammation, infection, serotonin dysregulation, bacterial overgrowth and neuroimmune mechanisms have been reported for IBS (5), and autoimmunity, inflammation and bile acid malabsorption in the case of MC (7). Associations with other diseases are seen in both disorders, MC with autoimmune disorders, e. g. coeliac disease, thyroiditis and rheumatoid arthritis, and IBS with depression and fibromyalgia (5, 7).

Recently, we have reported of autoantibodies against gonadotropin-releasing hormone (GnRH) in patients with IBS and dysmotility, whereas patients with inflammatory bowel diseases (IBD) did not express these antibodies (11). This has raised the hypothesis that GnRH antibodies are involved in the pathogenesis to gastrointestinal dysfunction. The

primary aim of this study was to compare gastrointestinal symptoms and subjective well-being in MC outpatients, with or without co-existing IBS-like symptoms, and assess to what extent these differed between the groups. The secondary aim was to examine the prevalence of serum antibodies against GnRH and its receptor in these groups.

Methods

The study protocol was approved by the Ethics Committee of Lund University, and all participants gave written informed consent when taking part in the study.

Patients

Women at the age of 73 years or younger, who had been treated for MC at any outpatient clinic of the Departments of Gastroenterology, Skåne, between 2002 and 2010 for MC, were identified by search for the ICD-10 classification for CC and LC. Only the 240 patients who had the diagnoses verified by colonic biopsy were invited to participate in the present study. Altogether, 159 (66.2 %) of the 240 invited patients were recruited to the study and 134 (55.8 %) patients also agreed to provide blood samples. One patient was excluded due to another IBD diagnosis a few weeks after the inclusion, leaving 158 (65.8 %) and 133 (55.4 %) patients, respectively, to be included in the final calculations and laboratory analyses. These patients represent the majority of cases of diagnosed MC in the most southern parts of Sweden. Both MC and IBS are more frequent in women than in men, and as the quality of life and experiences of symptoms differ between genders (1), we chose only to include women in the study.

Blood donors

Blood samples were collected from 98 consecutive blood donors (70 women, 28 men; median age 46 years; range 23–64) in April 2007 when they presented for regular blood donation at Skåne University Hospital. Serum was separated and stored at -20 ° C until analyzed.

Study Design

Between March and June 2011, invitations including study information and questionnaires about gastrointestinal symptoms and quality of life were sent by mail to all 240

women. They were also invited to visit the outpatient clinics of the Departments of Gastroenterology, Skåne University Hospital, Malmö or Central Hospital in Kristianstad, to provide blood samples for routine analyses at the Departments of Laboratory Medicine in Skåne at respective hospital. Antibodies against GnRH and a GnRH receptor peptide were analyzed by an ELISA assay. Questionnaires were completed one to three weeks before blood samples were collected. A reminding letter was sent a month after the invitation letter to those whom hadn't answered. Medical records were scrutinized, and age, gastrointestinal symptoms, duration of symptoms, examinations, and treatments were recorded.

Questionnaires

Gastrointestinal Symptom Rating Scale (GSRS)

The GSRS is a Swedish, disease-specific and self-administered questionnaire, designed to evaluate perceived severity of gastrointestinal symptoms during the previous week (12, 13, 14). The questionnaires include 15 items and use a 7-grade Likert scale. This gives a total range value between 15 and 105 where the highest score (seven) represent the most pronounced symptoms and the lowest (one) no symptoms. The items are divided into five dimensions representing Reflux Syndrome (two items), Abdominal Pain Syndrome (three items), Constipation Syndrome (three items), Indigestion Syndrome (four items) and Diarrhoea Syndrome (three items). Incomplete forms were excluded in the calculations.

Psychological General Well-being Index (PGWB)

The PGWB is a broad instrument to measure subjective well-being or distress during the previous week (15). The questionnaires include 22 items and use a 6-grade Likert scale. This gives a total range value between 22 and 132 where a low score (one) correspond to a poor level of well-being and the higher value, the better psychological well-being. The items are divided into six dimensions representing Anxiety (five items), Depressed mood

(three items), Positive well-being (four items), Self-control (three items), General health (three items) and Vitality (four items). Incomplete forms were excluded in the calculations.

Visual Analogue Scale for Irritable Bowel Syndrome (VAS-IBS)

The VAS-IBS is a short, psychometrical test developed to measure the treatment response and well-being during the last two weeks, in patients suffering from IBS (16). The questionnaires include nine items about gastrointestinal symptoms and psychological well-being. The seven items abdominal pain, diarrhoea, constipation, bloating and flatulence, vomiting and nausea, perception of mental well-being and the intestinal symptoms' effect on daily life used a scale from 0 to 100 mm, with 100 mm representing the best symptom/health. The two items urgency and feeling of incomplete evacuation of bowel passage were answered by "yes/no"- questions.

Rome III criteria

The patients completed a shortened version of the Rome III questionnaire, only including IBS symptoms (6). This questionnaire has been translated and validated into the Swedish language (M Simrén and Anna Rydén). Patients who fulfilled the criteria for Rome III were classified as also suffering from IBS symptoms.

Measurement of human antibodies against gonadotropin-releasing hormone (GnRH)

Blood samples were drawn from patients and serum was separated and kept frozen in -20° C until analyzed. Analysis of anti-GnRH antibodies was carried out by an ELISA method slightly modified from results described in a previous study (11). The wells of microtiter plates were coated with human GnRH-conjugated ovalbumin (OVA) or GnRH receptor-conjugated OVA ((NH₂)-MANSASPEQNQNHC SAINNSIPLMQGNLPY) (Innovagen, Lund, Sweden) in an overnight incubation at 4° C and thereafter the plastic wells were blocked with 0.5% bovine serum albumin (BSA) (A-7030, Sigma, St Louis, USA) in

PBS containing 0.05% Tween-20 (PBS-T). Dilutions of patient serum 1/400 in 1,0 µg OVA (A-5503, Sigma, St Louis, USA) / ml 0.5% BSA, PBS-T was then added to the plates and incubated for 2 h at room temperature (RT). After rinsing with PBS-T, deposition of autoantibodies directed to GnRH was detected using biotinylated rabbit anti-human IgM (673211, MP Biomedicals, Solon, OH, USA), IgG antibodies (ab7159, Abcam, Cambridge, MA, USA) or IgA antibodies (ab97218, Abcam, Cambridge, USA) appropriately diluted in PBS-T. After another incubation for 2 h at RT the plates were washed and the bound biotinylated antibodies detected by alkaline phosphatase-conjugated streptavidin (405211, Biolegend, San Diego, CA, USA), incubated for 1 h at RT. To develop a colour reaction a phosphatase substrate kit (37620, Pierce, Rockford, Ill, USA) was used. The absorbance at 405 nm was measured after 30 min of incubation at RT. Antibody levels are presented as relative units (RU) The cut-off value in the control group was defined using Receiver Operator Characteristics (ROC) curve. The antibody specificity was set to 97.5% (Table 1).

Statistical analyses

Statistical calculations were performed in SPSS, version 20.0 for Windows[®]. The scores of the GSRS, PGWB and VAS-IBS index were presented as median and interquartile ranges. The statistical significance of differences was determined by two-tailed Mann-Whitney U test or Fisher's exact test. $P < 0.05$ was considered to be statistically significant.

Results

Patient characteristics

In total, 158 women with MC were included in the study (median age 63 years, range; 27-73 years), CC was diagnosed in 92 (58%) patients (median age 64 years, range; 31-73 years) and LC in 66 (42%) patients (median age 63 years, range; 27-72 years). Of these 158, 87 (55%) patients also fulfilled the Rome III criteria for IBS (median age 63 years, range; 27-72 years), 49 (53%) of the patients with CC and 38 (58%) of the patients with LC (Figure 1). There was no difference in age between patients with or without IBS-like symptoms (median age 63 years, range; 27-73 years, and median age 64 years, range; 28-73 years, respectively, $p=0.134$). Neither was there any difference in disease duration between the two groups ($p=0.103$). Measurements of haemoglobin (Hb) in blood and C-reactive protein (CRP) in plasma were in the majority of patients within reference values, and did not reveal any differences in patients who fulfilled the Rome III criteria or not ($p=0.367$ and $p=0.307$, respectively).

Gastrointestinal symptoms and psychological well-being

Patients with IBS-like symptoms experienced much more symptoms and worse psychological well-being in all dimensions in GSRS and PGWB compared to patients without IBS symptoms (Table 2). There was no difference in symptoms between CC and LC when groups were compared independently of IBS-like symptoms (data not shown).

When performing subgroup analyses, the patients with CC and concomitant IBS-like symptoms had more gastrointestinal symptoms, without any differences in positive well-being and self-control, compared to patients without IBS symptoms (Table 2). Patients with LC had low scores in the reflux dimension, independently of IBS symptoms. All other items, except vitality, were affected by IBS symptoms (Table 2).

Constipation was a rare symptom in this cohort, and constipation was the only symptom on the VAS scale that did not differ between patients with or without IBS symptoms (Table 3).

In patients suffering from LC, concomitant IBS-like symptoms did not either affect the experience of bloating and positive well-being (Table 3).

Antibodies against gonadotropin-releasing hormone (GnRH) and its receptor

Altogether, anti-GnRH antibodies were detected in 11/133 (8.3%) MC patients and in 7/98 (7.1%) controls ($p=$. The prevalence of anti-GnRH receptor were 11/133 (8.3%) and 6/98 (6.1%) in MC patients and controls, respectively ($p=$ (Table 1). There was no significant difference in the prevalence of antibodies between CC or LC (data not shown). Half of the patients with antibodies suffered from IBS-like symptoms and half did not.

Discussion

There was a high prevalence of IBS-like symptoms (55%) in patients with MC, independently of CC or LC. Patients with concomitant MC and IBS symptoms had more severe gastrointestinal symptoms and worse psychological well-being than patients who did not fulfil the criteria for IBS. The differences could not be explained by disease activity or duration of disease.

The criteria for IBS are gastrointestinal complaints in the absence of organic changes (6). It is previously described that patients with IBS more often also suffer from MC when thoroughly examined (17). As the patients with MC have well-defined histopathologic findings, the term IBS-like symptoms should be used instead of IBS in these patients, in parallel with identified similar symptoms in patients suffering from IBD (2). Both groups may express intraepithelial lymphocytosis, but only CC express a thickened collagenous layer (8). Although different histological picture, the clinical pictures are similar in CC and LC patients with no difference between the two groups in symptom scoring.

Histologically, some studies have shown an increased number of mucosal T lymphocytes in IBS (18), whereas others have found normal T cell numbers in intestinal tissue (19). Normal histopathology may render severe gastrointestinal complaints with secondary impaired well-being and reduced quality of life in IBS patients (1, 7). This can be explained by two alternative explanations to perception of pain. Firstly, mechanosensitive primary gut afferents react to various stimuli by transmitting at elevated firing frequencies, which is interpreted centrally as nociception. Secondly, normally functioning afferents transmit accurate information, which is misinterpreted in processing centres of the spinal cord and cortical pain modulation circuits (20).

The higher prevalence of IBS-like symptoms in MC and in IBD compared to the general population (10-15%) may represent the peripheral component of pain perception due

to intestinal inflammation and thereby sensitization of afferent neurons (2, 5, 9, 10). However, the increased colonic sensitivity in idiopathic IBS is more influenced by a psychological tendency to report pain than neurosensory sensitivity (21). Irritable bowel syndrome does have a peripheral component, but the central components become more prominent as disease severity increases (22). As our patients were recruited from hospitals and not from the primary health care, we can suspect them to have a more severe disease (1). Thus, the histological picture may be of minor importance in IBS compared to cognitive and psychosocial functions (21), explaining the impaired psychological well-being and more pronounced symptoms in our IBS patients, although similar histopathology. The discussion above is provided that idiopathic IBS is the same as IBS associated with MC and IBD.

There was some discrepancy between the scores of GSRS and PGWB compared to the VAS-IBS scores. This may depend on that the VAS-IBS measures the last two weeks, while the other two questionnaires only measure the last week. Further, the VAS-IBS has been validated and tested in comparison to GSRS and PGWB questionnaires in greater patient cohorts with IBS, when MC and any other organic disease was excluded (16). In the present study with fewer patients, all patients also had MC, possibly obscuring the picture. The dimension with most discrepancy was constipation, a symptom more seldom present in MC. In GSRS and PGWB several questions are put together, whereas only one question is asked in the VAS-IBS each time, which can further influence the outcome. The MC patients in the present study had lower scores in all VAS-scales except constipation and nausea and vomiting compared to healthy controls, also when they did not fulfil the criteria for IBS (23).

In rats, GnRH and GnRH receptor have been found in ganglion cells of the myenteric plexus (24). The role of GnRH in the gut is not completely elucidated, but GnRH has been shown to inhibit gastric secretion and gastrin release in dog (25), to affect rat intestinal motility (26) and GnRH antibodies exert anti proliferative effects on gastrointestinal

tumor cell lines (27). The analogue leuprolide stimulates the hypothalamic-pituitary-gonadal axis, thereby down-modulating gonadal products, which are known neural antagonists of gastrointestinal motility (28, 29, 30). This may explain the effect seen by leuprolide in the treatment of functional bowel diseases (31), especially as a numerous expression of LH receptors have newly been described on myenteric neurons (32).

We have recently described the presence of antibodies against GnRH in patients with IBS and dysmotility, whereas patients with IBD did not express these antibodies (11). In the present study, we have expanded the analyses to include antibodies against GnRH receptor, to examine epitope spreading and molecular mimicry (33). Our finding that antibodies were not more prevalent in MC patients than in healthy controls, further supports the hypothesis that bowel diseases characterized by visible inflammation have another pathogenesis than bowel diseases characterized by pain and dysfunction. These findings also further underline the unspecificity of IBS symptoms. Although female predominance for both MC and IBS, antibodies against GnRH or its receptor do not seem to be involved in the aetiology to MC to the same extent as to idiopathic IBS. Nevertheless, addition of abdominal pain to MC patients impairs well-being and quality of life.

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Legend to Figure

Figure 1. Flow-chart over the patient population

