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Abnormal rectal endocrine cells in patients with irritable bowel syndrome

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ABSTRACT

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder. In a previous study the total number of endocrine cells in the rectum of IBS patients, as detected by chromogranin A, did not differ from that of healthy controls. While the total endocrine cell content of the rectum appears to be unchanged in IBS patients, changes in particular endocrine cells cannot be excluded. This study was undertaken, therefore, to investigate the cell density of different rectal endocrine cell types in (IBS) patients. Fifty patients with IBS (41 females and 9 males) were included in the study. Thirty patients had diarrhoea (IBS-D) and 20 had constipation (IBS-C) as the predominant symptom. Twenty-seven subjects were included as controls (19 females and 8 males). Rectal biopsy specimens were immunostained using the avidin–biotin-complex method for serotonin, peptide YY (PYY), pancreatic polypeptide (PP), and oxyntomodulin and somatostatin cells. The cell densities were quantified by computerised image analysis. The serotonin cell density did not differ significantly, although a type II statistical error cannot be excluded, due to the small size of the sample. The densities of PYY and Oxyntomodulin cells were significantly lower and that of somatostatin were significantly higher in IBS patients than controls. These abnormalities were observed in both IBS-D and IBS-C patients. The abnormalities in the endocrine cells observed in this study in the rectum differed considerably from those seen in the colon of IBS patients. This indicates that caution in using the rectum to represent the large intestine in these patients. These abnormalities could be primary (genetic) or secondary to changes in the gut hormones found in other segments of the gut and/or other pathological processes. Although the-cause-and effect relationship of the abnormalities found in rectal endocrine cells is difficult to elucidate, they might contribute to the symptoms associated with IBS. The densities of PYY and somatostatin cells are potential biomarkers with good sensitivity and specificity for the diagnosis of IBS.

1. Introduction

Irritable bowel syndrome (IBS) is a gastrointestinal chronic disorder characterised by abdominal discomfort or pain associated with altered bowel habits, and often bloating and/or abdominal distension [1,2]. IBS reportedly affects as many as 5–20% of individuals worldwide with an annual incidence of 196–260 per 100,000 [3]. While IBS is not known to be associated with the development of serious disease or with excess mortality, IBS considerably reduces the quality of life in IBS patients [3]. Besides the increased morbidity caused by IBS, it is an economic burden to society through increased sick leave and excessive consumption of healthcare resources, and other factors [3].

A previous study found that the chromogranin A cell density was reduced in the colon of IBS patients, and it has been suggested that this can be used as a marker in the diagnosis of IBS [4,5]. The colonic endocrine cell types that were affected were serotonin and peptide YY (PYY) cells [6]. The rectum contains the same endocrine cell types as the colon, namely serotonin, peptide YY (PYY), pancreatic polypeptide (PP), oxyntomodulin, and somatostatin-cells [6,7]. However, the rectum harbours a larger number of these endocrine cells and is more accessible for biopsies than the colon [7,8]. Surprisingly, a previous study found that the total number of endocrine cells in the rectum of IBS patients, as detected by chromogranin A, did not differ from that of healthy controls [9].

While the total endocrine cell content of the rectum appears to be unchanged in IBS patients, changes in particular endocrine cells cannot be excluded. This assumption is supported by the finding that changes in the serotonin cells do occur in the rectum of patients with IBS [10–14]. The present study investigated the various types of rectal endocrine cells in a cohort of patients with IBS comprising those with the

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diarrhoea-predominant (IBS-D) and constipation-predominant (IBS-C) subtypes. These subtypes were chosen in order to determine whether the wide difference in symptoms has an impact on the population endocrine cells.

2. Material and methods

2.1. Patients and controls

Fifty patients with IBS according to Rome Criteria III using the IBS module were included in the study [15]. These patients comprised 41 females and 9 males with a mean age of 37 years (range 18–64 years). Thirty patients had IBS-D and 20 had IBS-C. All of the patients had experienced their symptoms for many years and they could not associate the onset of IBS symptoms with any events, in particular gastrointestinal or other infections. All patients underwent a complete physical examination and were investigated by means of blood tests (full blood count, electrolytes, calcium and inflammatory markers), liver tests, and thyroid function tests.

Twenty-seven subjects that underwent colonoscopy with rectal biopsies were used as controls. Colonoscopy was performed in 20 of these subjects because of gastrointestinal bleeding, where the source of bleeding was identified as hemorrhoids ($n = 18$) or angiodysplasia ($n = 2$), and in 7 because of health worries caused by a relative(s) being diagnosed with colon carcinoma. These subjects comprised 19 females and 8 males with a mean age of 49 years (range 18–68 years). All of these subjects were healthy and had no gastrointestinal complaints other than those stated above. The study was performed in accordance with the Declaration of Helsinki and was approved by the local Committee for Medical Research Ethics. All subjects gave both oral and written consents for participating in the study.

2.2. Colonoscopy, histopathology and immunohistochemistry

Colonoscopy was performed on both patients and controls, and four biopsy samples were taken from the dorsal wall of the rectum about 15 cm from the anus. These biopsy samples were fixed overnight in 4% buffered paraformaldehyde, embedded in paraffin, and oriented during embedding in paraffin so that the biopsy samples were sectioned parallel to the crypt axis. The paraffin blocks were cut into 5-μm sections and were stained with haematoxylin–eosin, and immunostained by the avidin–biotin complex (ABC) method using the Vectastain ABC kit (Vector Laboratories) as described in detail elsewhere [16]. The primary antibodies used were monoclonal mouse anti-serotonin (Dako, code no. 5HT-209), polyclonal anti-porcine peptide YY (PYY; Alpha-diagnostica, code PYY 11A), polyclonal rabbit anti-synthetic human pancreatic polypeptide (PP; Diagnostic Biosystems, code no. #114), polyclonal rabbit anti-porcine oxyntomodulin (Acris Antibodies, code BP508) and polyclonal rabbit anti-synthetic human somatostatin (Dako, code no. A566). The antibodies were used at dilutions of 1:1,500, 1:1,000, 1:800, 1:400 and 1:200, respectively. The second layer biotinylated mouse anti-IgG and rabbit anti-IgG were obtained from Dako. Negative and positive controls were the same as those described elsewhere [16].

2.3. Computerised image analysis

The number of immunoreactive cells and the area of the epithelial cells were measured as described previously by using of Cell^D software (Olympus) [17]. A ×40 objective was used, for which each frame (field) displayed on the monitor represented an area of 0.14 mm^2 of the tissue in each field. Each individual and peptide hormone was measured in 10 randomly chosen fields. Immunostained sections from IBS patients and controls were coded and mixed, and all measurements were made by the same person (ME) without knowledge of the identity of the sections. The data from the fields were tabulated, the density of cells in the epithelium was computed and analysed statistically.

2.4. Statistical analysis

The gender difference between patients and controls was tested by Fisher's exact test, and the age difference was tested by Mann–Whitney non-parametric test. Multiple comparisons of the control, IBS-total (IBS as a whole), IBS-D and IBS-C patients were performed using the non-parametric-analysis of variance Kruskal–Wallis test with Dunn's test as a post-test. A probability value of $P < 0.05$ was considered to be indicative of statistical significance. The data are presented as median and interquartile range (25th and 75th percentile).

3. Results

3.1. Gender and age characteristics of patients and controls

The gender distribution did not differ between patients and controls ($P = 0.09$). However, the age distribution did differ significantly between patients and controls ($P = 0.002$).

3.2. Endoscopy, histopathology and immunohistochemistry

The colon and rectum were macroscopically normal in both the patients and the control subjects. Histopathological examination of the rectum biopsy samples obtained from patients and controls revealed normal histology. In the rectum of both patients and control subjects, serotonin-, PYY-, PP-, enteroglucagon- and somatostatin-immunoreactive cells were found mostly in the upper part of the crypts of Lieberkühn.

3.3. Computerised image analysis

3.3.1. Serotonin cell density

The serotonin cell density in the rectum of controls was 35 (24,56) cells/mm^2 (median and 25th and 75th percentile). The density of these cells in the IBS-total, IBS-D, and IBS-C groups were 32 (0.0,52), 26 (13,52), and 34 (0.0,52) cells/mm^2, respectively. There were no statistically significant differences in multiple comparisons between controls, IBS-total or the IBS subgroups ($P = 0.9$), or between controls and IBS-total, IBS-D and IBS-C ($P = 0.8$, 0.8 and 0.9, respectively) (Fig. 1).

3.3.2. PYY cell density

The PYY cell density in controls was 106 (92,139) cells/mm^2. The corresponding values for IBS-total, IBS-D and IBS-C were 58 (35,74), 58 (30,75) and 59 (34,74) cells/mm^2, respectively. The density of PYY cells did not differ significantly between IBS-D and IBS-C ($P = 0.6$),

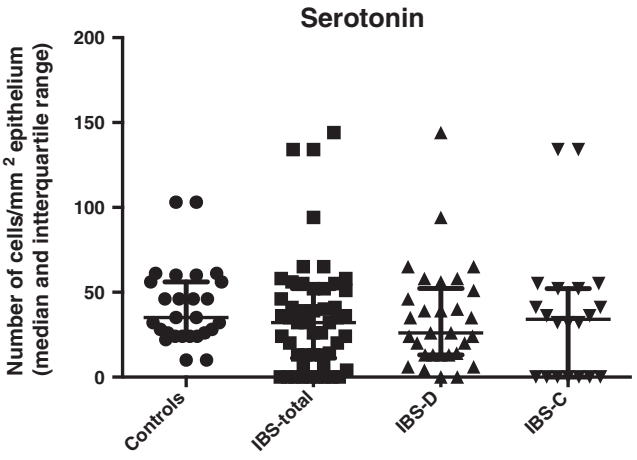

Fig. 1. Serotonin cell density in the rectum of controls, IBS patients as a whole (IBS-total), IBS-D and IBS-C.