**Scientific Research Process**

Irritable bowel syndrome (IBS) is a commonly diagnosed functional gastrointestinal disease. IBS is a typically recurrent disease characterized by abdominal pain or discomfort, stool irregularities, and bloating. A major subtype of IBS is IBS with diarrhea (IBS­D).

The pathogenesis of IBS-D is complex and poorly understood. Recent studies of the pathophysiology of IBS-D have focused on molecular mechanisms and have suggested that the levels of luminal and mucosal cytokines originating from the gastrointestinal tract may be altered in the gut of IBS-D patients. These alterations could result in dysregulation of gastrointestinal secretion and motility and an increase in visceral hypersensitivity. These cytokines can also affect the peripheral and central nervous systems and disrupt communication between the brain and gut.

Leptin is a 16 kDa non-glycosylated peptide hormone belonging to the type I cytokine superfamily. When bound to receptor sites, leptin not only exerts significant biological effects, such as appetite control, by signaling satiety and increasing energy expenditure, but also through modulation of the immune system and gastrointestinal function.

Few studies have specifically addressed the role of leptin in the pathogenesis of IBS. Therefore, the present study measured leptin expression in both the serum and intestinal mucosa of patients with IBS-D, and then analyzed the relationship of these levels with the clinical features, visceral sensitivity, and the number and activation rate of mast cells and nerve fibers in these patients.

We recruited 42 IBS-D patients (15 women and 27 men; mean age, 29.4 years; age range, 22-40 years) and 20 healthy controls (8 women and 12 men; mean age, 28.9 years; age range, 20-38 years). Patients and controls gave written informed consent before entry and the study protocol was approved by the Ethics Committee of the China-Japan Friendship Hospital (no. 2015-33) and conducted in accordance with the Declaration of Helsinki. On the day of entry, patients and healthy control subjects underwent assessment of clinical and psychological status, using validated questionnaires (including IBS Symptom Severity Scale, IBS-specific Quality of Life, Hamilton Anxiety Scale and Hamilton Depression Scale) along with visceral sensitivity test and venous blood collecting. On the second day, participants underwent colonoscopy after standard bowel preparation with 300g Polyethylene Glycol Electrolytes Power (Fortrans, BEAUFOUR IPSEN Industrie, Dreux, France) dissolved in 3 L of water and administered 6–7 h before endoscopy. In all subjects, we obtained 4 biopsies from the rectosigmoid junction in order to standardize the site of sampling. Of these, two biopsies were fixed in buffered 10% formalin and processed for Hematoxylin and eosin-staining (H&E) histology to exclude microscopic colitis and to perform mast cells staining and immunohistochemistry for leptin, leptin receptor, anti-protein gene product (PGP) 9.5 and tryptase. Another two biopsies were immediately soaked in reagent specified for quantitative real-time polymerase chain reaction (RNA Stabilization Reagent; QIAGEN GmbH, Hilden, Germany) and stored at -20℃ until assay. Serum leptin levels were assessed using enzyme-linked immunosorbent assay. Mucosal leptin expression and localization were evaluated by immunohistochemistry and immunofluorescence. Mucosal leptin mRNA levels were quantified by quantitative real-time reverse transcription polymerase chain reaction. Mast cell counts and activation rates were investigated with toluidine blue staining. Correlation analyses between these parameters were examined in patients.

All statistical analyses were performed using SPSS for Windows software, version 24.0 (SPSS Inc, Chicago, IL). All data were reported as mean values ± standard deviation (SD) or medians [interquartile range (IQR)]. Independent sample t-tests or nonparametric Mann–Whitney U-tests were used to analyze quantitative data. The χ2 test or Fisher’s exact test were used to analyze qualitative data. Correlations between two parameters were performed using Spearman’s correlation coefficient, followed by Bonferroni correction to adjust multiple comparisons, with a corrected significance level of 0.0038 (0.05/13). Two-tailed P values < 0.05 were considered significant.

IBS-D patients had significantly increased psychological symptoms and visceral hypersensitivity (P<0.001), and their mucosal leptin expression, leptin mRNA levels, and mast cell activation rates were significantly increased (P<0.05). Additionally, leptin expression was positively correlated with anxiety, depression, and the mast cell activation rate, but negatively correlated with the defecation sensation threshold and the maximum tolerance threshold during visceral sensitivity testing (adjusted P<0.0038).

This study presents evidence that leptin levels are increased in the intestinal mucosa of IBS-D patients, proposes a mechanism by which leptin may contribute to the pathophysiology of IBS, and provides some potential avenues for more specific and effective treatments in these patients.