https://doi.org/10.22416/1382-4376-2025-35-4-98-103
UDC 616.33/.34-008.87 + 616.33-008.3

Microbiota of the Stomach and Duodenum and Functional Dyspepsia: Is There Any Connection?

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Aim: to analyze the works published in the literature on the possible association of symptoms of functional dyspepsia (FD) with changes in the microbiota of the stomach and duodenum.
Key points. The data published in the literature indicate that there are significant differences between the composition of the microbiota of the stomach and duodenum in patients with FD and in healthy individuals. It is believed that changes in this composition can lead to an impairment of the integrity of the gastroduodenal mucosa with subsequent effects on the main pathogenetic factors of FD. Some studies have shown the effectiveness of probiotics in the treatment of FD patients. At the same time, the insufficient evidence base of the results does not currently allow us to give them an unambiguous assessment.
Conclusion. The relationship between changes in the microbiota of the stomach and duodenum requires further research.
Keywords: microbiota of the stomach and duodenum, functional dyspepsia, probiotics
Conflict of interest: the authors declare no conflict of interests.

For citation: Sheptulin A.A., Kardasheva S.S., Kurbatova A.A. Microbiota of the Stomach and Duodenum and Functional Dyspepsia: Is There Any Connection? Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2025;35(4):98–103. https://doi.org/10.22416/1382-4376-2025-35-4-98-103

Микробиота желудка и двенадцатиперстной кишки и функциональная диспепсия: есть ли какая-то связь?

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Цель обзора: провести анализ опубликованных в литературе работ о возможной связи функциональной диспепсии (ФД) с изменениями микробиоты желудка и двенадцатиперстной кишки.
Основные положения. Опубликованные в литературе данные свидетельствуют о наличии существенных различий между составом микробиоты желудка и двенадцатиперстной кишки у больных ФД и здоровых лиц. Полагают, что изменения этого состава могут приводить к нарушению целостности гастродуоденальной слизистой оболочки с последующим влиянием на основные патогенетические звенья ФД, способствуя висцеральной гиперчувствительности, нарушению аккомодации желудка после еды, его замедленному опорожнению. В некоторых работах показана эффективность применения пробиотиков в лечении больных ФД. В то же время недостаточная доказательная база полученных результатов не позволяет дать их однозначную оценку.
Заключение. Связь между изменениями микробиоты желудка и двенадцатиперстной кишки и патогенезом ФД, а также значение пробиотиков в лечении ФД требуют дальнейших исследований.
Ключевые слова: микробиота желудка и двенадцатиперстной кишки, функциональная диспепсия, пробиотики
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования: Шептулин А.А., Кардашева С.С., Курбатова А.А. Микробиота желудка и двенадцатиперстной кишки и функциональная диспепсия: есть ли какая-то связь? Российский журнал гастроэнтерологии, гепатологии, колопроктологии. 2025;35(4):98–103. https://doi.org/10.22416/1382-4376-2025-35-4-98-103

Functional dyspepsia (FD) is one of the most common functional disorders associated with impaired interaction between the brain and the gastrointestinal tract. FD is manifested by pain and a burning sensation in the epigastric region, as well as a feeling of fullness in the epigastric region after eating and early satiety (postprandial distress syndrome). The leading pathogenetic mechanisms of this disease are considered to be hypersecretion of hydrochloric acid, visceral hypersensitivity and motility disorders of the stomach and duodenum [1].

The Rome IV diagnostic criteria for disorders of gut-brain interaction, published in 2016, do not consider dysbiosis of the stomach and duodenum microbiota to be a possible etiological factor of FD [1]. However, studies published in recent years indicate that changes in the microbiota of the upper gastrointestinal tract may play an important role in the development of FD.

Features of the microbiota of the stomach and duodenum in functional dyspepsia

The density of bacteria in the intestinal contents is typically 10^{12}/g, whereas the number of bacteria in the stomach is very low — at around 10^3 colony-forming units per 1 mL of gastric fluid. This is associated with the inability of many microorganisms to survive in such a highly acidic environment [2].

The most common bacteria found in the stomach are members of the *Streptococcus* and *Prevotella* genera, accounting for around 50 % of all bacteria detected in gastric fluid [3]. H. Nakae et al. [4] compared the microbiota composition of the liquid stomach contents in 44 FD patients and 44 healthy control subjects. The stomach contents were collected in the morning on an empty stomach. In patients with FD, the bacterial content of the collected fluid was significantly lower than in the control group. An inverse correlation was also found between the content of *Prevotella* bacteria and the severity of symptoms of postprandial distress syndrome. M. Igarashi et al. [5] demonstrated that in patients with FD the gastric fluid microbiota was dominated by *Bacteroidetes* bacteria rather than *Proteobacteria*, with no presence of *Acidobacteria*. By contrast, the control group of healthy people showed a quantitative predominance of *Proteobacteria* over *Bacteroidetes* and the presence of *Acidobacteria*.

L. Zhong et al. [6] conducted a pilot study assessing the microbiome associated with the duodenal mucosa and found that patients with FD had increased levels of *Streptococcus* bacteria and decreased levels of *Prevotella*, *Veillonella* and *Actinomyces* bacteria compared to healthy individuals in the control group. The total microbial content on the duodenal mucosa correlated with the severity of dyspepsia symptoms associated with food intake and quality of life. According to the authors, this indicates the potential involvement of changes in the duodenal microbiome in FD pathogenesis.

E.R. Shanahan et al. [7] used 16S ribosomal RNA sequencing to study the composition of the microbiota associated with the duodenal mucosa in 56 FD patients and 30 healthy control subjects. Gastric emptying time and dietary characteristics were also assessed. The study revealed a link between FD symptoms and the predominance of *Firmicutes*, *Bacteroidetes*, and *Fusobacteria* in the microbiota associated with the duodenal mucosa. An inverse correlation was also found between the relative abundance of *Streptococcus*, *Prevotella* and *Veillonella* spp. and gastric emptying time. However, no association was found between the duodenal microbiota profile and the dietary characteristics of patients.

A. Fukui et al. [8] studied microbiota associated with mucous membranes of various parts of the upper gastrointestinal tract. During oesophagogastroduodenoscopy, biopsies were taken from patients with FD and healthy individuals in the control group from the oral cavity, the middle third of the oesophagus, the body and antrum of the stomach, and the descending part of the duodenum. The microbiota profile was studied using 16S ribosomal RNA sequencing. FD symptoms were assessed using a questionnaire. No significant differences in the structure of the microbiota associated with the mucous membrane were found when assessing α-biodiversity between FD patients and the control group. However, differences were found when assessing β-biodiversity. An increase in the content of *Firmicutes* microorganisms was noted in all mucosal samples from FD patients. At the genus level, FD patients had a higher relative abundance of *Streptococcus* bacteria than the control group, which correlated with the presence of dyspepsia symptoms in all samples.

The composition of the mucosal microbiota in the proximal part of the duodenum of patients with dyspeptic symptoms, as determined by 16S ribosomal RNA sequencing, depended on the presence or absence of *H. pylori* infection. At the species level, the microbiota associated with the duodenal mucosa was dominated by *Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria* and *Fusobacteria*, and at the genus level — by *Ralstonia*, *Streptococcus*, *Pseudomonas*, *Haemophilus*, *Herbaspirillum*, *Neisseria* and *Veillonella*. In terms of biodiversity, the α-biodiversity of the microbiota was

more pronounced in *H. pylori*-infected individuals. Significant differences were found in β-biodiversity between the groups. The relative content of *Haemophilus, Neisseria*, *Prevotella pallens*, *Prevotella 7* and *Streptococcus* was higher in individuals infected with *H. pylori* [9].

W. Wang et al. showed that the number of bacteria colonising the gastric mucosa and their biodiversity are significantly reduced in patients with FD infected with *H. pylori*. Eradication therapy increased the biodiversity of bacteria colonising the mucous membrane and promoted the growth of probiotic strains, such as *Leuconostoc mesenteroides*. The authors concluded that eradicating *H. pylori* contributes to restoring microbial diversity and promoting favourable shifts in gastric microflora composition [10].

Probiotics in the treatment of functional dyspepsia

Several studies have evaluated the effectiveness of probiotics in treating FD. For instance, H. Nakae et al. [4] administered yogurt containing the probiotic *Lactobacillus gasseri OLL2716* (*LG21* strain) to patients with FD for 12 weeks. During treatment, the severity of dyspeptic symptoms characteristic of postprandial distress syndrome decreased and the initial low level of *Prevotella* bacteria increased, approaching that of the control group. The authors proposed considering this increase as a potential biomarker for the effectiveness of FD treatment. They also suggested that one possible mechanism of action of this probiotic may be an antisecretory effect, given that intragastric pH levels increased significantly during *LG21* treatment. The positive effects of treating FD patients with the *LG21* probiotic strain were also noted by other authors [5].

T. Ohtsu et al. [11] conducted a double-blind, randomised, placebo-controlled study evaluating the results of 12-weeks treatment for FD patients not infected with *H. pylori*, using yoghurt containing the *LG21* probiotic (main group) and yoghurt without it (control group). The frequency with which the four main symptoms of FD (fullness in the epigastric region after eating, early satiety, epigastric pain and a burning sensation in the epigastric region) were eliminated was 35.3 % in the main group and 17.3 % in the control group ($p = 0.048$).

A. Takagi et al. [12] investigated the efficacy of a 12-week probiotic *LG21* treatment for 131 *H. pylori*-infected FD patients, compared to 67 patients who received a placebo. After treatment, the severity of the feeling of fullness in the epigastrium after eating in patients of the main group was significantly less than before treatment ($p < 0.05$). In this group, after a course of probiotics, there were significantly fewer patients who had maximum assessments of the bloating symptom according to the visual analogue scale ($p < 0.05$). No differences in the frequency of side effects were found in either group.

The effect of the probiotic strain *LG21* on gastric emptying was studied in a double-blind, randomized, placebo-controlled trial in patients with mild to moderate gastric emptying disorders. At the screening stage, delayed gastric emptying in the participants selected for the study was confirmed using a special breathing test with octanoic acid labeled with the ^{13}C isotope — the study included patients with a peak gastric emptying rate (T_{max}) of more than 55 minutes after ingestion of liquid food. A pronounced delay in gastric emptying ($T_{max} \geq 75$ minutes) was an exclusion criterion. Fourteen patients received yogurt containing probiotic *LG21*, and 14 patients in the control group received yogurt without probiotic. After 12 weeks of treatment, gastric emptying improved in 9 of the 14 patients in the main group and in 4 of the 14 patients in the control group (odds ratio (OR): 4.1). However, the authors concluded that the reliability of this finding must be confirmed by a study involving a larger number of participants [13].

Other probiotics were also used in the treatment of FD. For example, E. Sun et al. [14] conducted an open study in which they used the probiotic *Lactobacillus paracasei* (strain *LC37*) in 26 patients with FD to evaluate its ability to influence the severity of FD symptoms. The composition of the intestinal microbiota was determined using 16S ribosomal RNA sequencing. Short-chain fatty acids and metabolites in feces were also evaluated before and after a four-week course of treatment. The results showed that the severity of abdominal pain and belching decreased significantly on day 14 of treatment and had almost completely disappeared by day 28. Additionally, the abundance of beneficial bacteria, including *Lactobacillus*, *Lactococcus* and *Weissella*, increased, while the abundance of pathogenic *Lachnoclostridium* bacteria decreased. An increase in short-chain fatty acids and positive changes in metabolome have been demonstrated.

L. Wauters et al. [15] conducted a randomised, double-blind, placebo-controlled pilot study investigating the efficacy of eight weeks of treatment with the probiotics *Bacillus coagulans MY01* and *Bacillus subtilis MY02* in 68 FD patients receiving proton pump inhibitors. 32 patients were assigned to receive probiotics, while 36 patients received a placebo. A higher proportion of patients responded to treatment in the probiotic group

than in the placebo group (48 and 20 %, respectively; $p = 0.028$). The incidence of side effects was similar in both groups.

L. Drago et al. [16] used a combination of probiotics *Lacticaseibacillus rhamnosus, Lactiplantibacillus pentosus, Lactiplantibacillus plantarum* and *Lactobacillus delbrueckii* subsp. in 2676 patients with FD (1357 — with postprandial distress syndrome and 1319 — with epigastric pain syndrome) for 30 days. The probiotic combination was administered either as monotherapy or together with other drugs (prokinetics, antacids and proton pump inhibitors). The dynamics of clinical symptoms were assessed before the start of treatment and 15 days after its completion. A decrease in the severity of clinical symptoms was noted in all patients, both those receiving probiotics alone and probiotics along with traditional medications. At the same time, with postprandial distress syndrome on the background of probiotic monotherapy, the heaviness in the epigastric region after eating and early satiety completely disappeared in more patients than in the groups with combined therapy. In patients with epigastric pain syndrome, there were no differences between the groups of probiotic monotherapy and combined treatment in the frequency of symptoms of dyspepsia.

Nevertheless, the effectiveness of probiotics in the treatment of patients with FD requires further research. J. Zhang et al. [17] have shown in a systematic review and meta-analysis that probiotics and prebiotics generally have a positive effect on FD symptoms. However, an analysis of four randomized controlled trials with probiotics alone did not demonstrate a significant positive effect in reducing the severity of dyspepsia symptoms: relative risk — 1.13; 95 % CI: 0.99–1.28; low degree of heterogeneity between studies ($I^2 = 0$ %; $p = 0.67$).

Pathogenesis of functional dyspepsia and microbiota of the stomach and duodenum

However, the role of disturbances in the microbiota of the stomach and duodenum in the development of FD remains insufficiently studied. It is believed that the reflux of intestinal contents, including bile and bacteria from the small intestine (in particular *Escherichia coli*), into the duodenum and stomach may disrupt the integrity of the gastroduodenal mucosa. This subsequently influences the main pathogenetic mechanisms of FD, contributing to visceral hypersensitivity, impaired gastric accommodation after eating and delayed gastric emptying [2, 5].

The results of a vote held during an online consensus meeting of the European Society of Neurogastroenterology and Motility in 2020, which was dedicated to FD, are indicative. The meeting was attended by 42 experts from 25 countries. A statement was considered accepted if it was voted for (in full or with minor restrictions) by more than 80 % of experts [18].

Statement 4.11 of this consensus meeting contained: “Altered duodenal microbiota composition is a pathophysiological mechanism in functional dyspepsia”.

This statement was not accepted (“overall agreement” — 34 %; “agree strongly” — 10 %; “agree with minor reservation” — 24 %; “agree with major reservation” — 39 %; “disagree with minor reservation” — 12 %; “disagree with major reservation” — 15 %).

It can be assumed that such results of the expert vote can be explained by the lack of convincing evidence regarding the role of gastric and intestinal microbiota dysbiosis in FD pathogenesis. The effectiveness of probiotics in treating FD is also unclear. One can agree with the opinion of G. Tziatzios et al. [19] that the results of probiotic treatment for FD cannot currently be considered reliable, as many studies did not consider *H. pylori* infection status. According to the Kyoto Consensus on *H. pylori*-associated gastritis, FD can only be diagnosed in patients who do not have this infection, or who continue to experience dyspeptic symptoms after successful eradication [20].

Therefore, analysing the possible relationship between changes in stomach and duodenal microbiota and FD, we can conclude that, despite significant interest in this topic, further research is required as it remains insufficiently studied.

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Sheptulin A.A., Kardasheva S.S., and Kurbatova A.A. made an equal contribution to the development of the concept and formulation of the aim of the review, collection and processing of materials, writing and editing the text and proof checking.

Вклад авторов

Шептулин А.А., Кардашева С.С. и Курбатова А.А. внесли равный вклад в разработку концепции и формулирование цели обзора, сбор и обработку материалов, написание и редактирование текста и проверку верстки.

Submitted: 14.04.2024 Accepted: 01.08.2025 Published: 29.08.2025
Поступила: 14.04.2024 Принята: 01.08.2025 Опубликована: 29.08.2025